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AEROSOL LOADED TOROIDAL VORTICES FOR ENHANCED OCULAR DRUG DELIVERY

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AEROSOL LOADED TOROIDAL VORTICES FOR ENHANCED OCULAR DRUG DELIVERY

by

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Dedication

To my mother, Sandie Herpin, for raising me with unconditional love and support.

And

To the loving memory of my father, Lenis C. Herpin.

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AEROSOL LOADED TOROIDAL VORTICES FOR ENHANCED OCULAR DRUG DELIVERY

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Over that last few decades there has been an increase in the creation of new medicinal agents to treat various ophthalmic diseases, from small molecule drugs to highly targeted biologics. While these advances in drug discovery have enabled a new wave of therapies, many problems regarding their accurate, reproducible and safe administration still remain. The standard vehicle for topical ocular drug delivery is the eye drop, and despite its popularity, there are some significant limitations to their use. It is estimated that in many cases less than 5% of the active reaches the target tissues. While the remainder of the dose is either spilled out onto surrounding tissue or it is rapidly drained through the lacrimal ducts where it can be absorbed into the systemic circulation. Current drug delivery technologies have focused on improving bioavailability and patient compliance, and it is expected that further improvements can be made in these areas by incorporating the use of precision drug loaded aerosol vortices. Within the framework of this dissertation, two main aspects of a novel ophthalmic aerosol drug delivery device were investigated; the mechanical features that dictate the dose delivery, and the formulation aspects that control the characteristics of the aerosols that are being delivered. In early studies, investigations into the mechanism of the dose deposition were explored in order to gain knowledge into predicting the performance and

to tune the delivery of a wide range of therapeutic concentrations. In later studies, after the device and dosing characteristics where established, studies were conducted on different formulation strategies in order to incorporate active pharmaceutical ingredients that would otherwise have unfavorable physicochemical characteristics for incorporation into an aqueous based system. These studies included the use of solubilizing agents and their effect on the characteristics of the aerosol generated from the device, as well as a novel particle engineering technology that could be utilized to incorporate the use of nanoparticles or colloidal particulates into the device or for other uses. The use of these formulation techniques thereby increases scope of therapeutic agents that can be incorporated for use in the device, further improving its therapeutic potential.

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INTRODUCTION

The role of pharmaceutical scientists, engineers and physicians in designing new ocular drug delivery systems as whole, is to improve the safety, tolerability and convenience for patients in hopes to achieve superior therapeutic outcomes and improve quality of life. Over the last few decades pharmaceutical scientists have made great advances in solving some of the major problems associated with delivering medicine to the desired ocular tissues. However, many of these problems still remain due to the complex anatomical barriers present in the ocular system. The internal components of the eye are largely protected from the bloodstream by the blood-retina barrier and the blood-aqueous barrier. While a select few drugs can pass through these barriers a majority cannot. Often times, extraordinarily high blood levels, sometimes toxic levels, must be achieved in order to get the drug to partition into the tissues of the eye. It is for these reasons the eye is rarely treated via the oral/systemic route. Delivering medications topically to the front of the eye, while better suited than the systemic route, still has major barriers. The major drug delivery barrier for topical administration is the cornea. More specifically, the corneal epithelial layer represents the largest permeation barrier to entry into the eye, and is the rate limiting barrier to most hydrophilic drugs (1).

An overwhelming majority of prescribed medications for treating front of the eye diseases are dispensed as solutions in standard multi-use eye-droppers. Eye drops have

been used in treating the eyes for over 100 years and have major advantages in terms of expense, ease of manufacturing and general patient acceptance. Despite their prevalence, eye drops have some major limitations and risks associated with their use, especially when used for a prolonged period of time or in vulnerable patient populations such as pediatrics or geriatrics (2-4).

LIMITATIONS OF THE EYE DROP:

Formulation aspects:

Topical instillation of liquids into the eye requires that the physiological features of the eye are not severely disrupted. The eye is highly regulated in terms of pH, osmolarity, lipid content, ionic strength, protective mucins etc. When adding a substantial volume into the eye, the tear film is entirely disrupted and diluted with the instilled vehicle. Therefore eye drop formulations must be sterilized, stable and osmotically balanced (171 -1711 mOsm/kg). But because some drugs need to be formulated at high concentrations that would normally exceed the isotonic levels, it is more important to establish the sterility and purity before adjusting tonicity. Also, the pH of eye-drops must be within range of 2 - 11.5 as to not damage or burn the surface of the eye and elicit a foreign body response and thus increase lacrimation and clearance (5). In order to avoid this response, promote retention and maximize absorption, the preparation must be formulated between pH 7.0 and 7.7. In addition the buffering capacity of the system must be quite low as to not overtake the endogenous restoration of the tear film, which in many cases in unavoidable. Unfortunately, many of the topical ophthalmic agents are alkaloids, such as pilocarpine, have been found to be chemically unstable in alkaline solutions so they must be formulated at slightly lower pH of between pH 4 - 5

(6). They must also be formulated with a lower buffer capacity as to not overwhelm the natural buffering system in the eye. Furthermore, the tear film contains many different enzymes, some of which could degrade labile drugs from within the tear film. Finally, eye-drop solutions must be sterilized and manufactured under cGMP conditions and because they are intended as multi-use aqueous based systems, the formulation must contain preservatives to prevent microbial growth or be packaged into disposable single use blisters. And many of these preservatives have been shown to have corneal toxicity with prolonged use (7-10).

Dropper Bottle Aspects:

The eye dropper bottle itself, contributes to the large amount of error associated with eye drop administration. Various manufacturers have designed different bottles made of different types of plastic with different nozzle geometries and performance. Different plastics provide more or less rigidity and require different amounts of pressure to dispense a drop. In addition, the pressure required to generate drops is also dependent on the nozzle diameter. Smaller diameter nozzles require more force to produce drops and have a slower rate of production, while large nozzles require less force and can create droplets rapidly with ease (11, 12). All of these factors play a role in drop size/weight uniformity. The physicochemical characteristics of the formulation and how they interact with specific nozzles also plays a role. The principle behind Tate's Law is that the surface tension of the formulation and the nozzle diameter both play a key role in the mass/diameter of droplets produced. However, in a real word settings patient factors often come into play. Sklubalova and coworkers have extensively investigated several other factors that contribute to the dose variability of eye drops. They found that the

dispensing angle is also a major contributor to the size of the drops produced. Other factors they mentioned were: temperature, surface tension of the formulation, specifically related to benzalkonium chloride concentration, and rate at which the drops were dispensed. In one tip design they measured drops to be 60% less than what the theoretical maximum weights predicted by Tate's Law would be, indicating the complexity of the effects of nozzle design and its interaction with different formulation characteristic (13, 14) . In other studies done German et al. with the Minums® dropper, they found very large variability in dispensing doses with the same nozzle. Because these nozzles are manufactured by injection molding, the authors suspect there was very little deviation in geometry and the variability was associated with the inherent nozzle design and the different interactions between the plastic and the formulation (15). All of these studies highlight the importance of co-developing a formulation side by side with the intended dropper to help avoid problems downstream.

PHYSIOLOGICAL LIMITATIONS:

The stable human tear film contains approximately 7 µL of fluid and the pre-corneal cul-de-sac can transiently contain up to 30 µL before blinking. The problem is that the volume of typical eye drops ranges between 50 – 75 µL (16). Upon instillation, a large portion the liquid can spill out of the precorneal area onto the surrounding tissue or can be rapidly drained through the puncta and down through the nasolacrimal ducts. From there the drug can be absorbed into the systemic circulation and metabolized or excreted. Beyond the over-flow, afferent nerves innervated into the ocular surface are capable of detecting transient changes in environmental conditions, such as: pH, carbonic acid levels and fluid levels. Upon activation, these nerves send signals to the efferent

parasympathetic nervous system which stimulates the lacrimal gland to produce tears (17). This is known as reflex tearing and depending on the strength of the signal, it can cause a disproportionate amount of drainage in attempts to clear the surface and re-establish homeostasis. Ultimately, this results in a very low residence time for drugs instilled into the precorneal film. It has been estimated that the instilled drug is cleared from the front of the eye in less than 5 minutes (18). Because of this clearance rate, in order to increase the dose, an additional drop would need to be instilled outside of the five minute window because any additional fluid added would not alter the concentration at the surface, it would only replace the fluid residing with the same concentration. This subsequent drop time limitation has been shown to be a major source of error in the scaling of doses in terms of bioavailability and even efficacy in some cases. Furthermore, this dosing problem becomes more troublesome when incorporating a second eye drop medication. Because the initial dose causes an increase in tear production/drainage, the second instilled medication will have a diminished residence time. In addition, this second medication further dilutes the initially instilled eye drop, ultimately causing both to be less effective (19). The evidence provided by Chrai et al. strongly supports the use of combination drug products (i.e. incorporating two drugs in a single solution) in cases where multiple drugs are necessary for treatment.

Anatomical Barriers:

Delivering medications from the front of the eye into the interior structures requires overcoming several barriers, both from a physicochemical and structural perspective. The first barrier protecting the front of the eye is the tear film. The tear film is comprised of a lipid layer floating on top of an aqueous layer. This lipid layer serves

to both lubricate the surface of the eye between blinks but its main role is to limit or reduce evaporation of the aqueous layer. The next layer is the aqueous layer which contains a multitude of water soluble entities such as ions, proteins/enzymes and mucins. The aqueous layer serves many different roles in maintaining the health of the corneal cells, including replenishing nutrients and maintaining hydration. The aqueous layer also presents a barrier to the solubilization capacity for poorly water soluble drugs. Insoluble drugs delivered as suspensions must first pass into solution before they can be absorbed through the cornea, and often times these molecules cannot establish a high enough concentration to be absorbed sufficiently because of their low aqueous phase solubility.

While there are several routes of entry into the anterior of the eye, they can basically be broken down in two main routes: corneal and non-corneal. Entry through the cornea represents a unique challenge in comparison other biological membranes or structures, in that it is comprised of three alternating multi-phasic layers (i.e. lipophilic-hydrophilic-lipophilic). In addition, the apical cells are connected via tight junction structures (zonula occludens) which often severely limit paracellular transport, therefore a large portion of drug transport processes rely on transcellular mechanisms. The outermost layer, the epithelium, is lipophilic and allows for more oily compounds to penetrate. The middle layer of the cornea is the stroma. It is approximately 450 μm thick and comprised of multiple hydrophilic plates of collagen fibrils, and contributes to 90% of the corneal thickness (20). Finally, the innermost layer, the endothelium, is lipophilic but it is also formed in conjunction with the Buchman's membrane. This serves as the last physical barrier before entering in the aqueous humor. Because of this multi-phasic nature, certain molecules will have advantages over others and a balance between lipophilicity and hydrophilicity must be in order so that the molecule can transition through the layers with the least resistance. Barring the occurrence of transporters and

permeation enhancers, this permeation rate is almost entirely based on the physicochemical properties of the drug molecule itself.

Non-corneal entry is bit less direct in that it includes direct penetration through the conjunctiva and sclera and into the anterior uvea, where it can diffuse throughout the other tissues. The apical layer of the conjunctiva is also more permeable to hydrophilic molecules as well as some peptides or macromolecules, and due to looser junctions it can facilitate more paracellular transport. The non-corneal surface area is roughly 20 times larger than that of the cornea and can allow for the transport of drug molecules that would normally not penetrate the cornea (21). However, this absorption is also still severely limited because it is in concurrent competition with the ocular and conjunctival vasculature for loss via systemic circulation (22).

PATIENT COMPLIANCE/ADHERENCE:

Social and Psychological Barriers:

Patient compliance is a well known contributor to the success or failure of an ophthalmic drug therapy. The patient compliance aspect is especially magnified in cases where chronic or lifelong therapy is necessary for success. After analyzing several large datasets from pharmacy claims, Friedman et al. proposed the actual adherence to glaucoma treatment is below 50% (23). Of the contributors to patient compliance/adherence, the main contributors fall into two main categories: voluntary non-compliance and involuntary noncompliance. In a study conducted by Konstas et al., 44% of patients were not compliant with their glaucoma medication to a point where it became clinically significant. Of the 44%, 29% were voluntary and 15% were involuntary non-compliance (24).

Voluntary non-compliance in centered around the patient's attitude towards a therapy or the social circumstances in which the patient chooses to not take the medication they already acquired. It also includes circumstantial situations, including lifestyle matters that inhibit their usage. Some of the most often cited reasons for voluntary patient noncompliance are: forgetfulness due to complicated dosing regimens, situational events which disrupt scheduling, costs and health provider convenience, low health literacy, and lack of perceived disease severity (25). While it was cited that simplifying dosing regimens could improve outcomes, from a pharmaceutical development perspective, outside of significantly altering dose potency and duration, very little can be done to alleviate the behavioral problems (26). It is important to stress the significance of patient education from healthcare professionals in order to equip patients with the correct mindset while undergoing therapy. Improving communication between patient and prescriber is a well established means to improve rates of adherence and with technical advances in therapy the two can work synergistically for optimize patient outcomes (24).

The other source of noncompliance, involuntary compliance also known as dyscompliance, relates specifically to patients who willfully comply with dosing regimens however they cannot practically administer the medications correctly. In these situations, very little can be done from an educational perspective; however vigilance in assessing and monitoring proper patient use of devices to ensure they are competent in their use. A majority of the most highly cited sources of error with administration are related to the use of eye drops in particular. Some of the most common difficulties are associated with proper squeezing the dropper bottle, especially in geriatric populations. Often times, in these cases more than one drop can be dispensed. In addition, patients also reported difficulty seeing the dropper bottle tip and therefore had trouble aligning

and allowing the droplet to fall into the eye, and in some cases touching the dropper tip to the eye (27-29). It is in these arenas pharmaceutical scientists and engineers can develop novel devices and dosage forms in order to make accurate dosing more convenient, especially for those with physical limitations.

STRATEGIES FOR IMPROVING TOPICAL OCULAR DRUG DELIVERY

A majority of the strategies used to improve ocular bioavailability are based on increasing the residence time in the precorneal film, increasing the drug concentration or the incorporation of permeation enhancers. However, in some cases it may not be feasible to increase to the concentration or include permeation enhancers due to toxicological effects (30). Therefore, the safest approach is to increase the tear film residence time. One way to increase residence time is to increase the viscosity of the instilled liquid, by incorporating hydrophilic polymers or other viscous liquids (31). Improving residence time can also be achieved by using microparticulates and by incorporating some bioadhesive polymers to entangle the mucin layer of the corneal epithelium. The downside of these techniques is that many times these solutions/suspensions can cause blurring of the vision and can be irritating to the patient. In addition, special care must be taken to ensure particulates are smaller than 10 μm in order to eliminate possible corneal abrasions. The other strategy is to reduce tear production, thus reducing drainage and elimination of the drug from the ocular surface. While it has been established that many different mechanisms including, temperature, pH, and tonicity can irritate the ocular surface and instigate tearing, the eye is also sensitive to the volume of the liquid in the pre-corneal space.

Instilled Volume and Retention in the Tear Film

In a landmark paper, Chrai et al. investigated tear film turnover rates in rabbits as well as the effect that different instilled volumes have on the drainage rates (16). In order to investigate tear film residence, they conducted non-sampling lacrimal fluid turnover tests using radioactive technetium colloids. They instilled the radioactive technetium solution in different volumes and immediately began collecting data. As can be seen in Figure 1.1, the fraction of the remaining tracer for 50 μL instillation volume falls considerably fast within the first few minutes, while the 5 μL drops have a much more gradual decline. At 25 minutes 50% of the 5 μL dose still remains, while only about 10% remains for the 50 μL drop (16). The authors suggest that the explanation for the disparity is that two competing drainage mechanisms are occurring simultaneously, rapid drainage of the instilled volume and natural basal tear turnover. So in the case of the larger volume, the rapid decline is due to drainage and the second phase or plateau is due to the tear turnover. In the case of the small volume, the initial drainage is drastically reduced and the predominating clearance is done by the basal tear turnover. This idea is further supported because of the intermediate clearance rates are also observed at with intermediate instillation volumes.

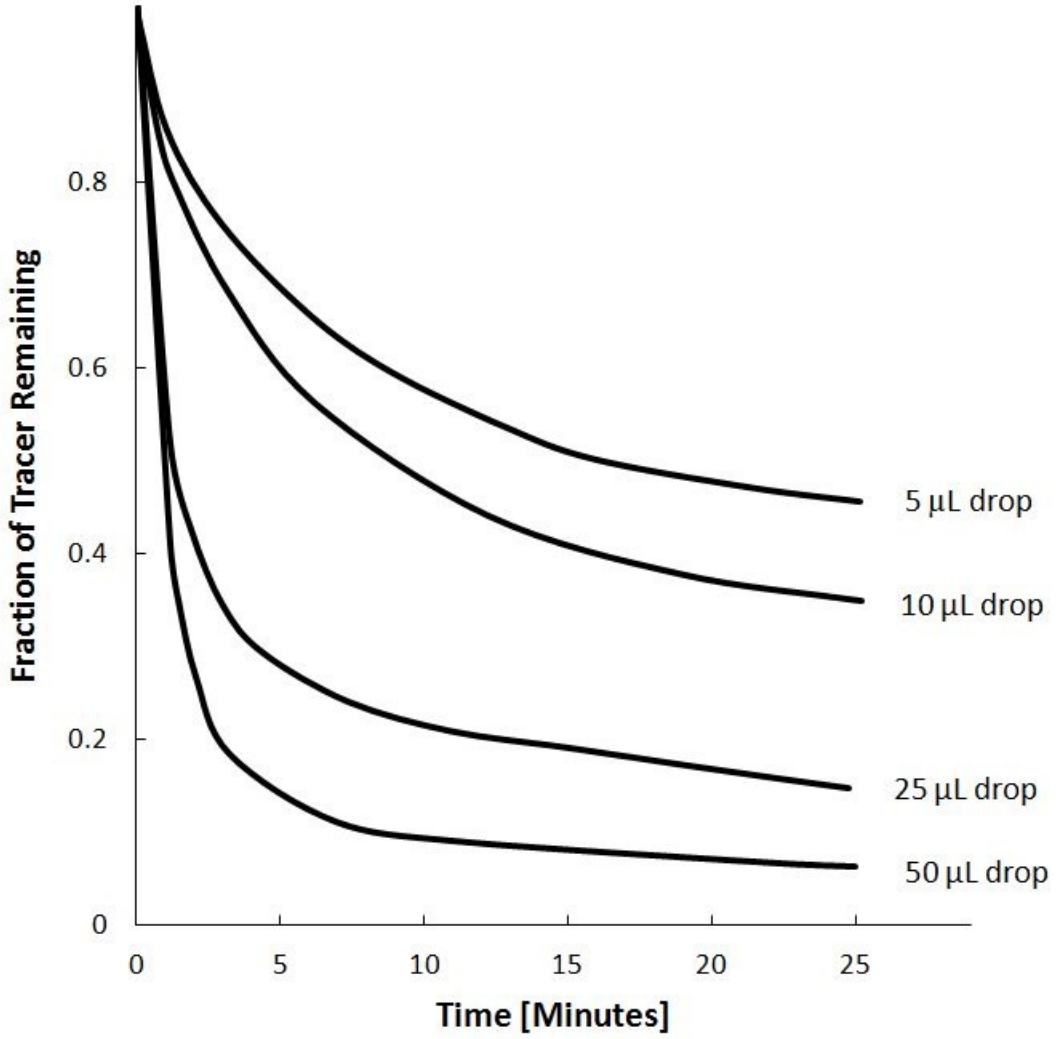

Figure 1.1. Decreasing the dosing volume of instilled eye drops showed large improvements in tear film retention over time. Adapted from Chrai et al, 1973

Drop Volume and Bioavailability

As follows from the improved retention time, Chrai et al. also demonstrated that this small volume effect also translates to improved ocular bioavailability by measuring in-vivo activity. They measured the pupil miosis from a fixed dose of 0.136 mg of Pilocarpine Nitrate administered at different concentrations and volumes. They compared instilled volumes of 5, 10, 25, 50 and 75 μL and they found that the 5 μL drops had double the peak effect compared to the 75 μL, and the AUC was ~4 fold that of the 75 μL.

It has long been known that one could improve the retention in the front of the eye by increasing the viscosity of the vehicle as in ointments and gels, but this was of the first times it was demonstrated that by simply reducing the instilled volume it would improve the residence time without altering the structure of the drug or adding permeation enhancers to improve permeability. Therefore, these findings were very important because they offered a new simple way to improve efficacy without increasing exposure or dramatically altering the formulation.

Bioavailability: Competing Drug Permeability vs Drainage Rate

Many years later, Keister et al. mathematically described the relationship between the time dependent integral of drug tear film concentration and flux across the corneal membrane. And by using the known first order decay for the tear film, it was shown that the maximal tear film concentration over time could be achieved by instilling a virtual "zero" volume drop (32). Figure 1.2 shows that when a drug has a lower permeability and a fast drainage rate, there is a zero-drop effect. The zero-drop effect is the theoretical

maximum bioavailability that can be achieved due to minimizing the instilled volume. As the instilled volume approaches zero (μLs), the effects of induced drainage and clearance are minimized. Furthermore, the degree at which the zero-volume effect occurs is highly related to the physico-chemical properties of the drug, and the interactions of the drug with local physiology. In cases where the drug is highly permeable and drainage from surface is low, the volume of the instilled drop does not have an effect on the absorption. This is the theoretical best case scenario, however it is practically very rarely observed (without nasolacrimal occlusion, which is discussed elsewhere (33)). The most common case would be where the drug has intermediate permeability and an intermediate or variable drainage rate. Finally, at this point in time, it is has been well established that instilled volumes larger than the stable tear film instigate tear production rate and the transient dilution in the tear film by nearly doubles its normal rate (34, 35).

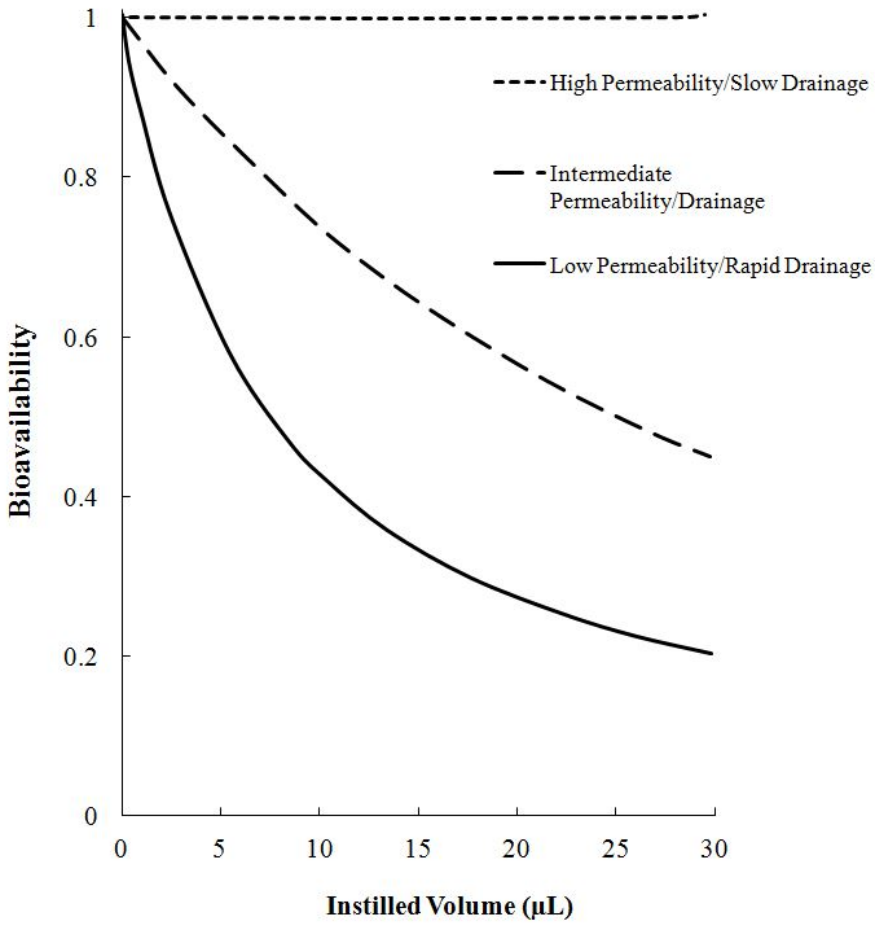

Figure 1.2. Theoretical relationship between ocular bioavailability and instilled drop volume when controlling for dose. The drug drainage rate and permeability are a major influence in the ocular bioavailability. Adapted from Keister et al.,1991

Environmental and Pharmacological Effects on Tear Film Retention

The baseline level of tear production is approximately 0.5 μL- 3 μL/minute, this means that typical tear turnover should be between 5 and 30 minutes (36). However because of complexity in the physiological conditions and variability between patients the effects of tear production can be quite large. Physical/chemical irritation and/or emotional factors can cause reflex tear production to increase from 3 to 400 μL (34, 37). In addition, transient changes in temperature or relative humidity can also affect basal tear production/turnover. All of these various factors, both predictable and not, can affect the clearance of the drug from the surface of eye. For example, the topical administration of decongestants causes vasoconstriction of the capillaries in the eyes in order to reduce redness. But when this vasoconstriction occurs in the cavernous body of cannicula in the nasolacrimal ducts, it causes a reduction in extracellular tissue volume which then causes the opening of the duct way lumen. In this scenario, the addition of the drug molecule itself pharmacologically exacerbates or alters the drainage from the precorneal film (38, 39). Conversely, some ophthalmic drugs that are irritating can produce a foreign body response which can simultaneously increase tear production and cause constriction of the drainage ducts causing highly variable changes in precorneal film retention. Therefore, due to the dynamic and many times unpredictable changes in basal tear drainage or clearance it would be pragmatic to reduce drug instillation volume to improve bioavailability and reduce variability whenever possible (35, 40, 41).

PREVIOUS INVESTIGATIONS INTO UTILIZING SMALL DROP VOLUMES OR SPRAYS:

Halberg et al. conducted early trials in humans for the use of topical ophthalmic sprays as an alternative means to administer glaucoma medicine to the eye. Patients were given 1 drop of a 1, 2 or 4% pilocarpine in one eye and the same concentration of the spray in the other eye. The drop in intra-ocular pressure was comparable to between the spray and drop at each time point, and after 120 minutes both eyes saw similar declines in activity. They also conducted a similar study over the course of the 3 months and the sprays were shown to control the intraocular pressure as well as the drops (42).

Since then several groups have investigated the use of topical ocular sprays for several different applications. Akman et al. showed equal pupil dilation between a 1% spray of tropicamide and an eye drops, but with less discomfort than the drops (43). Several others researchers have investigated sprays for use as cycloplegics and mydriatics for pediatric applications because of the routine nature of eye exams and the potential for decrease systemic effects (44-49). In addition, it has been well established that the medicine instilled in the eyes can rapidly absorb into the systemic circulation and cause systemic effects. However, recently Alpay et al. reported two case studies where excess eye drops spilled out of the eyes and on the eye lids and face, where they cause extreme discoloration. There was clear evidence of percutaneous absorption indicated by localized pale regions where vasoconstriction occurred (Figure 1.3, below). Neonates are especially vulnerable to the systemic effects of the commonly used mydriatic drugs due to having a decreased or immature metabolism and small body weight but at this age their epidermis is not quite fully developed (50). Special care must be taken to ensure excess drug is wiped away from the skin to limit the risks for systemic exposure. Furthermore, researchers suggest minimizing drop volume and performing nasolacrimal occlusion to further reduce any risks (49, 51).

Figure 1.3. Skin paleness around the eyes due to overflow and vasoconstriction from mydriatic eye drops. (Alpay et al., 2010)

CURRENT TECHNOLOGIES FOR DELIVERING SMALL VOLUMES TO THE EYE

Small Volume Dosage Forms and Formulation Strategies:

Delivering small volumes into the ocular space has shown to be a tedious and technical task, especially when dealing with low viscosity liquids. Standard dropper bottles are not capable of delivering micro drops because they are limited by the geometry of the nozzle as well as the surface tension and rheology of the formulation (52). In addition there is a practical limit in the reduction in droplet size from formulation approaches and in many cases it may not be optimal to alter the formulation in hopes to reduce the droplet size because of some other less desirable effect on the tolerance of the formulation. For the purposes of this manuscript, non-liquids (i.e. semi-solids) will be considered as they do not contribute excess liquids into the ocular cavity. They may however contribute the foreign body response on a case by case basis.

Ophthalmic Ointments:

One popular dosage for topical delivery is the ophthalmic ointment. Ointments are effective for two main reasons. They are able to drastically improve the residence time in the precorneal space and they are generally well tolerated. In some cases they may even be soothing to topical inflammation. While topical ophthalmic ointments are beneficial for improving residence which leads to improved bioavailability, they have a few drawbacks to their regular use. Ointments are well known to cause blurred vision, which can lead to poor patient compliance. Furthermore, because of their semisolid nature are prone to mishandling and dosing inaccuracies (53, 54). Finally, due to the

inherent increased contact time, the patient will also have increased exposure to preservatives, many of which have been shown to have toxicities.

Semifluorinated Alkanes (SFAs):

Beyond using aqueous based vehicles, researchers have been investigating a new class of compounds with properties that are quite favorable for ophthalmic use, Semifluorinated alkanes(SFAs). Semifluorinated alkanes are molecules that have a perfluorocarbon chain attached to a hydrocarbon chain. Ophthalmologists have successfully used SFAs as tamponade agents during complex retinal surgeries, and they have been shown to be chemically and physiologically inert and have very low surface tensions (55). When formulating aqueous based vehicles there is a practical limit the reduction in the size of the droplet produced. This is due to the limitation of the effectiveness of surfactants and other excipients in further reducing the surface tension. When formulating with SFAs, not only is there in inherent reduction in surface tension allowing for smaller droplet formation from the SFA, they are also quite good solvents for a large number of drug substances(56). In addition, they have comparable refractive index to that of water, so vision blurring would be minimized. Furthermore, Dutescu et al. also found that SFAs were adequate solvents for cyclosporine and were superior to the brand product, Restasis® (ophthalmic emulsion) in promoting in-vitro permeability of cyclosporine into the anterior chamber. Based on predictions done by Wiederholt et al., the concentrations achieved could be therapeutically relevant for treating internal inflammation(57). While in the case of dry eye syndrome, Novaliq®, a company based off of SFA technology currently has several products currently in development and in various stages of clinical trials.

OPHTHALMIC INSERTS- ERODIBLE/DISSOLVING

Novel Ocular Delivery System (NODS):

A more recent advance in the area of improving ophthalmic retention and bioavailability is the use of insertable drug loaded water soluble films. The NODS films were developed by Chauvin Pharmaceuticals Ltd., and overcome some of the problems associated with previous ocular inserts such as the Ocursert®. Earlier inserts suffered from problems of local irritation and they would commonly be ejected from palpebral fissure. The NODS units are different in that they are very small and flat so they can easily be inserted into the conjunctival sac with the applicator. Once inserted the water soluble polymers begin to hydrate/dissolve and release the drug dispersion. NODS have been demonstrated to be as effective in delivering tropicamide to that of drops but had a much longer duration. NODS have also been evaluated for use with chloramphenicol and pilocarpine. They offer a particular benefit for patients who have difficulty administering eye drops and can be an effective delivery system for immediate and controlled release (58).

Lyophilates:

Similar to the NODS system, Steinfeld and Lux et al. developed the use of a drug loaded solid lyophilate which is designed to be inserted into the conjunctival sac of the lower eye lid (Figure 1.4). The lyophilate can be made with hydroxypropyl methylcellose and the active ingredient or they can be made with neat drug. All solids/powders are intended to dissolve into the pre-existing tears and form high concentration gradient. This dosage form is administered to the prebulbar fissure via a

matchstick sized applicator. Upon blinking the powder disperse into the tear film where they are dissolved at very high concentrations. In a pilot study using fluorescein, scientists were able to see a 4 fold increase precorneal film retention and showed an increase in corneal permeability as assessed by the increased anterior chamber concentration compared to the eye drops (34, 59). In addition to lyophilates others have investigated the use of dry powders for topical administration to the eye. Hardarson et al. demonstrated that timolol dry powder formulations were well tolerated in the rabbit eyes, and showed that this dosage form/delivery method could provide a simplified means to avoid the use of toxic preservatives found in aqueous based eye drops(60).

Figure 1.4. Patient applying solid lyophilate to the conjunctival sac with an applicator (Lux, 2014)

Nanowafers

Researchers at Baylor College of Medicine have been investigating the use of these customized microfabricated nanowafers for use in topical ophthalmic administration. The fabrication of these wafers begins with using e-beam lithography to generate 500 nm wells into the surface of a silicon wafer to form a master template. From there, a poly (dimethylsiloxan) positive imprint is cast. This imprint is then used to create polyvinyl acetate mold with approximately 500 nm wide divots. Finally a polymer drug solution is then loaded into the wells. In one experiment, doxycycline wafers were applied topically to anesthetized mice. Researchers were able to detect high levels of the drug in the tear film after 2 hrs and detected its presence in the cornea for up to 24 hrs using intravital confocal microscopy (61). Furthermore, this research group has also investigated a large number of peptides and biologics for use in this system. This technology appears to be very promising because they have observed increased permeability and increased residence time in the front of the eye, and because it utilizes materials that have already been established as safe are commonly used in the eye this technology should translate easily into clinical trials (61).

NON-ERODIBLE INSERTS:

Ocusert ® Alza Corp.,CA, USA

One of the first modified release dosage forms for use in the eye is the Ocusert® Device. This dosage form is comprised of pilocarpine HCl mixed with alginate gel mixture formed into a rod-type geometry and has drug release rate controlling membrane surrounding the drug mixture. The tube like geometry is then connected end to end to form a soft white circular ring. This is used by placing into the inferior cul-de-sac (lower eyelid). This device can be worn continuously throughout the day to alleviate the need for applying multiple doses to the eyes.

CONTACT LENSES

Vistakon® Drug loaded contact lenses:

Contact lenses have been widely investigated in their role as a drug delivery vehicle for few different reasons. For one, they have been shown to be well tolerated in many different patient populations and can be self administered. Fundamentally, contact lenses are a biocompatible hydrogel which is an excellent material to contain and offer prolonged release of an active compound. The use of contact lenses as drug loaded carriers solves a couple key problems with ocular drug delivery. Mainly the use of drug loaded contact lenses obviates the need for frequent dosing with eye drops which leads to better compliance. In addition the steady state release of the drug offers a more consistent pharmacokinetic profile and reduces errors associated with improper patient dosing. Several researchers and a couple companies have investigated their use with several different drugs such as: timolol, dexamethasone, and various beta-blockers,

antihistamines and antimicrobials for treating front of the eye diseases (62, 63). There are currently no commercially available drug loaded contact lenses on the market, however, Vistakon® Pharmaceuticals (a Division of Johnson and Johnson) has previously conducted a couple Phase III clinical trials for safety and efficacy for ketotifen loaded lenses. In the safety study, the drug loaded lenses did not show any statistically significant differences in irritation or makers for toxicity to that of the blank contact lens (64, 65). Also, non drug loaded approaches have been investigated as well. In 2014, SEED Company Pte Ltd looked into the effects of alginic acid loaded contact lenses for the treatment of Dry Eye (66).

Intracanalicular Depot

One type of system that operates very similar to punctal plugs is the intracanalicular depot developed by Ocular Therapeutix™, Inc. This dosage form is comprised of proprietary polyethylene glycol (PEG) hydrogel polymer matrix which is loaded with drug and inserted into the lacrimal duct. Upon coming in contact with tear fluid, the implant swells and holds itself tightly within the duct. Over time the devices erodes while drug diffuses outwards toward the tear film for a sustained effect. This device offers a couple unique advantages over other punctal type systems. Firstly, this device is composed of a swellable smart polymer which expands to hold the device in place as opposed to having an anchor type system used in other technologies. Also, because it is inserted beyond the puncta orifice, it is invisible from a cosmetic perspective. Another unique feature is that because the device can't be seen from the outside, patients can confirm its presence using a special light which illuminates a

fluorescent tag from within dosage form. Finally, a major advantage of this type of system is that the dosage form erodes and is washed out through the lacrimal system once the dose has been administered. Other systems require removal from specialized health care personnel.

In a recent clinical study assessing reduction in intraocular pressure, a 90 day supply of travoprost supplied by the implant was comparable to timolol drops applied twice daily with a blank punctal plug used for masking purposes (67). In addition to glaucoma and ocular hypertension, this device is also being assessed for its potential in delivering dexamethasone for treating Dry Eye, Chronic Allergic Conjunctivitis, as well as ocular inflammation and pain associated with cataract surgery (68-70).

Punctal Plugs

One type of a drug eluting punctal plug system originally investigated by QLT Inc., and now being developed by Mati Therapeutics Inc., The Evolute®, consists of a drug loaded core that is encapsulated in either a plunger or L-shaped silicone geometry. This specific geometry was designed in order to enhance retention in the puncta after it is inserted into the lower puncta with a proprietary tool. The use of a punctal plug type system overcomes many of the limitations of eye drops, and other types of inserts. This system eliminates the need for preservatives and reduces patient compliance and adherence effects of eye drops. Furthermore, this system is not cosmetically noticeable, does not interfere with patient vision. Finally, this plug does not make prolonged contact with the corneal epithelia as in the case with contact lenses which thereby reduces risks for corneal abrasions or irritation. In a recent phase II clinical trial with latanoprost,

results showed a consistent reduction of intraocular pressure of about 5 mmHg over 12 week period with a very high ocular retention rate of 92% and very few serious adverse events (71, 72). Some of the drawbacks of this system from a formulation aspect are that because of the small geometric size of the system there is a limitation in the maximal dose loading available. This restricts the therapeutic uses to either highly potent compounds for prolonged effects or to less potent drugs for a shorter duration. From a patient's perspective, the major drawback is that it requires a doctor's visit for administration and in a small number of patients excessive lacrimation was observed. Future clinical trials are being planned for use in treating ocular allergies and inflammation in addition to a phase III trial for glaucoma with latanoprost (73).

There are also non-drug loaded punctal inserts designed specifically to obstruct tear flow and drainage, such as the Parasol® Punctual Occluder. These devices can be used to treat symptoms of Dry Eye in cases when root cause is where tear production is lower than normal. In addition, there are also specialized occluders which have small channels within them to allow for passage of some tear fluid but to overall serve to assist in allowing tear film accumulation. Finally, plugs can be used in conjunction with other topically instilled solutions in some cases. Studies have also shown that use of topical cyclosporine eye drops can have an additive benefit in treating dry eye above what either the drops or the plugs have been shown to do alone (74).

DRUG DELIVERY DEVICES/COMBINATION PRODUCTS:

Compliance/Coordination Devices:

Over the last several years there have been a large number of devices to assist in the instillation of the eye drops. These devices for the most part assist patients in aligning the dropper tip over the eye and/or in the depression of the bottle for patients who have difficulty squeezing the bottle to produce a single drop. One example, the Xal-Ease™ (Pfizer Ophthalmics, New York, York), allows the patient to the load a prescription eye-drop bottle of Xalatan ® (0.005% latanoprost) into the device and it is equipped with an attachment to assist in removing the eye dropper lid to reduce possible contamination. The patient can then align the eyepiece of the device around the ocular orbital and administer the medication from a horizontal position by squeezing an actuator trigger as depicted in Figure 1.5.

In a previous clinical trial, patients much preferred this device over standard drops and the outcomes of the results showed no clinical significant difference between the dropper bottle and the device (75, 76). While this device does not significantly reduce the droplet size that is instilled per say, it does however, help to ensure that patients are able to administer the proper the number of drops and deposit them properly (77). One of the downsides to the Xal-Ease device is that it is specially designed to only hold the Xalatan dropper bottles. There are however, other devices aimed at being more universal such as the Auto-drop® and the Easy Drop®. And for use in patients with limited dexterity or with rheumatoid arthritis there have been others introduced such as the Opticare Eye Drop Dispenser® and Opticare Ortho® shown in Figure 1.6. Another device that works in a slightly different method than the previous administration aids and is available over-the-counter, is the SimplyTouch® eye-drop applicator. This device

allows the patient to place a drop from their existing bottle into small divot or reservoir at the end of a soft plastic applicator Figure 1.7. The droplet is held in place by surface tension and the patient can then touch the droplet to the eye and it is quickly wicked away. This device appears to overcome many of the technical difficulties with administration and would also help to reduce wasting from over squeezing dropper bottles, however there may still be some concerns with cleanliness with re-use as well as possible damage done to the surface of the eye if incorrect contact is made. Furthermore, because the applicator relies on a fixed volume for administration variances in formulation composition can possibly fill the reservoir to different levels depending on surface tension, viscosity density etc. For simple formulations, this applicator appears to be promising.

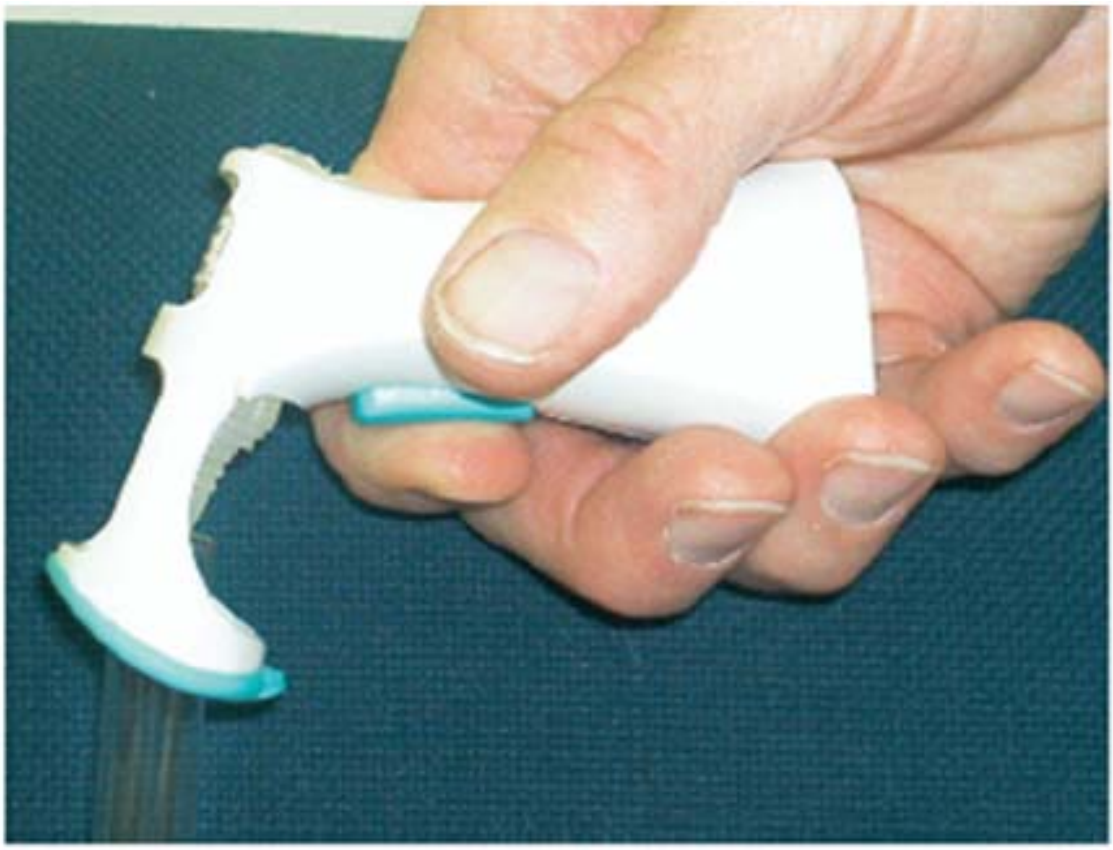

Figure 1.5. Xal-Ease® containing a bottle of Xalatan® be actuated into a test tube for analysis (Semes, 2007)

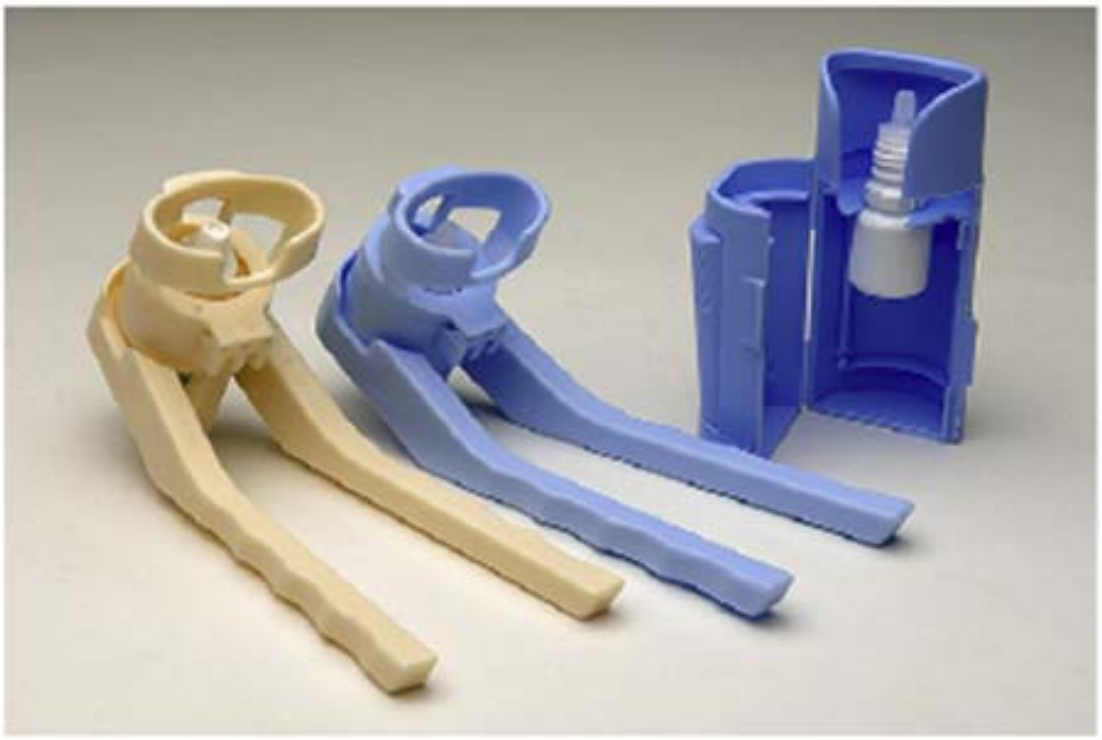

Figure 1.6. Versatile eye drop dispensers for patients with limited dexterity. Opticare Ortho ® (left) and Opticare ® , The Eye Drop Dispenser ® (right). (Get Permission from Cameron Graham, Ltd.) (Programs to optimize adherence in glaucoma. (Kowing et al, 2010)

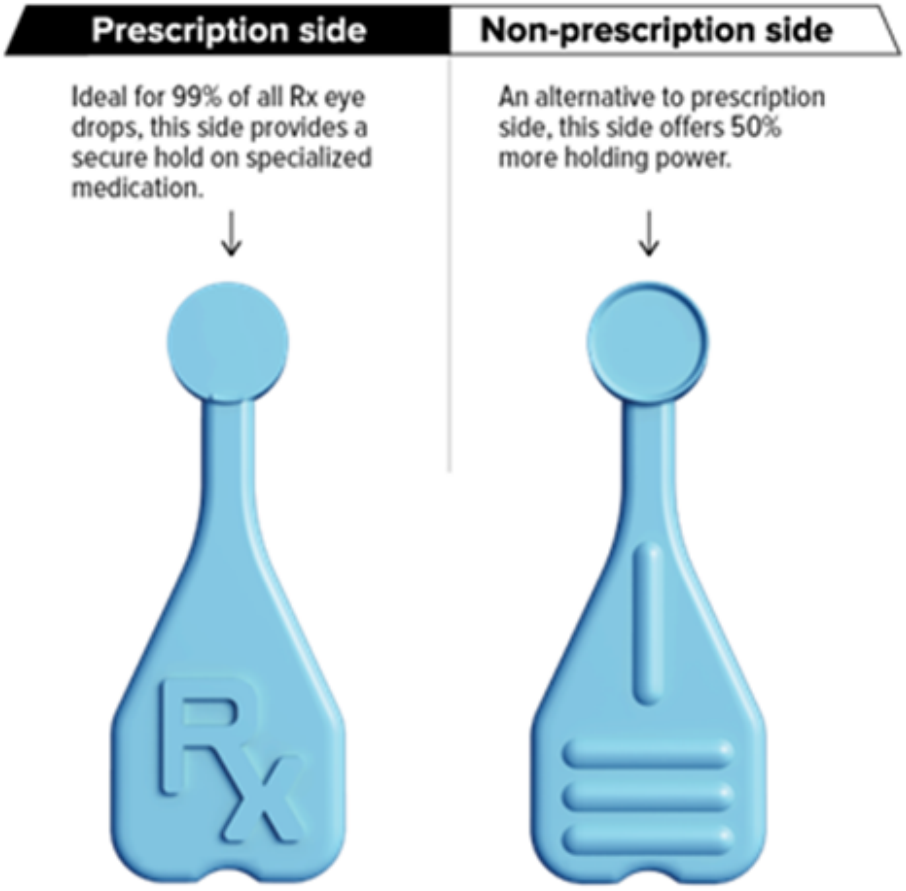

Figure 1.7. SimplyTouch® Eye Drop Applicator. The prescription side serves as a stage to hold the eye drop, while the other side has a reservoir to hold large non-medicated drops.

METERED/MICRO-DROPPER DEVICES

µ-Drop™

The mu-drop technology is a cartridge or blister type system that allows patients to load a blister containing a certain number of doses into a dispensing unit. This unit contains an internal needle which punctures the blister allowing the internal drug solution to pass down a narrow channel. The inventors of this technology found that by applying a set amount of force onto the blister via the actuator, they can produce very reproducible droplet volumes. Furthermore, they found a direct correlation between needle/channel diameter and droplet size. By utilizing such a small channels, the authors claim this device is capable of producing droplets in the 2 -25 µL range (78).

In a recent proof of concept clinical trial involving 30 healthy volunteers, the effect of droplet size on pupil dilation was assessed using the topical mydriatic, tropicamide. In addition to measuring pupil size ten times over a 2 hr period, the patients filled out a questionnaire to assess any adverse effects. One group received a micro-drop of 2.4 µL in both eyes while the other group received a standard drop, 38 µL. In both cases a 0.5% tropicamide solution. After a 7 day wash out the other drop was administered and the same measurements and questionnaire were used (79).

Scientists found that non-inferior mydriasis could be achieved using the micro-drop, which had 15 times less volume than the standard drop, and all of the patients experience pupil dilation adequate for general fundoscopy. Furthermore, according to the questionnaire, patients experienced few adverse effects and a large reduction in complaints of irritation/discomfort in the micro-drops vs. the standard drops (79).

Eyeinstill™ Device (MED*Instill*™, *CT, USA)*

MedInstill Technologies has created an Intact ™ one-way visco-elastic valve that allows fluids to be dispensed while serving as a non-contamination barrier. The concept of the valve is based around how the valves in the arteries of the cardiovascular system operate. The inventors claim that the valve allows for dispensing accurate, reproducible volumes of liquid formulations as low as 20 μL, all while preventing outside contaminants from entering. This technology could have major benefits in formulating multi-use drops without preservatives, especially for those which have been shown to have toxicities (7-9).

Novelia®- Sterile multi-dose dropper (Numera, France)

The only currently marketed preservative free multi-dose system commercialized in the US is the Novelia® device. This device prevents the ingress of contaminants using the PureFlow™ technology. The one-way valve can fit onto many different types of bottles and is capable of dispensing suspensions and high viscosity liquids.

IONTOPHORETIC DEVICES/SYSTEMS

Recently, several new iontophoresis technologies have been coming down the development pipeline. All of these technologies have the same basic operational concept in that, and electric field is used to migrate selectively ionized (or polar) molecules across key membranes, including the cornea, sclera and choroid, and even into the retina (80). While the dosing will require a doctor's visit, this technology has a major advantage over

injectable or implantable dosage forms, which have an increased risk of hemorrhage, retinal detachment or infection. This non-invasive technique has been used with large number of corticosteroids and antibiotics, as well as macromolecules and dendrimers or small nanoparticles (81-84). The actives/particles can be loaded in a solution or gel which applied to dosing chamber which makes contact with the ocular surface via different geometries depending on the application and device.

Polymer/Hydrogel Based Systems

The OcuPhor™ hydrogel (Iomed Inc., Salt Lake City, USA) is made of a polyacetal sponge which is loaded with a drug formulation and an electrode which can be placed in the inferior cul-de-sac adjacent to the sclera, and is then controlled by an external dosing device controller. Another similar device is the Visulex ™ (Aciont Inc., Salt Lake City, UT, USA). This device has a couple unique features that differentiate it from others. It has an optimized scleral shaped geometry to avoid exposing the surrounding tissue to the drug, thus preventing loss and systemic effects, and an in-situ drug depot mechanism. The Visulex ™ device imparts electrical energy onto the drug ions and the counter-ions while in solution, causing them to both penetrate into the target tissue, where they are joined together and form a precipitate. This precipitate will have reduced permeability and thus can act as a reservoir for a sustained effect (80). Furthermore, proof of concept work was done in a rabbit model demonstrating that the depot effect can also occur in the back of the eye with a small molecule and also with macromolecules (82, 85).

The Eyegate® II System (Waltham, MA, USA)

The Eyegate® II System uses a ring shaped silicone eyepiece that is designed to go around the cornea and only make contact with the sclera for trans-scleral delivery, and is capable of delivering medications to both the anterior and posterior segments of the eye (Figure 1.8). When comparing the amount of drug absorbed from frequent eye-drop instillation compared to iontophoresis using the Eyegate® II, a single transcorneal iontophoretic session of 1 minute @ 1 mA showed a 30 fold levels of dexamethasone phosphate in the cornea(81, 86). The flagship drug(lead clinical compound) for use in this combination product is EGP-437(dexamethasone phosphate) was granted orphan status in January 2009 for use in corneal graft rejection and has since been evaluated in various clinical trials for different indications including: anterior uveitis, dry eye syndrome and for use in cataract surgery with intraocular lens replacement (87, 88). Furthermore, early phase clinical trials are underway for its use in treating macular edema (89).

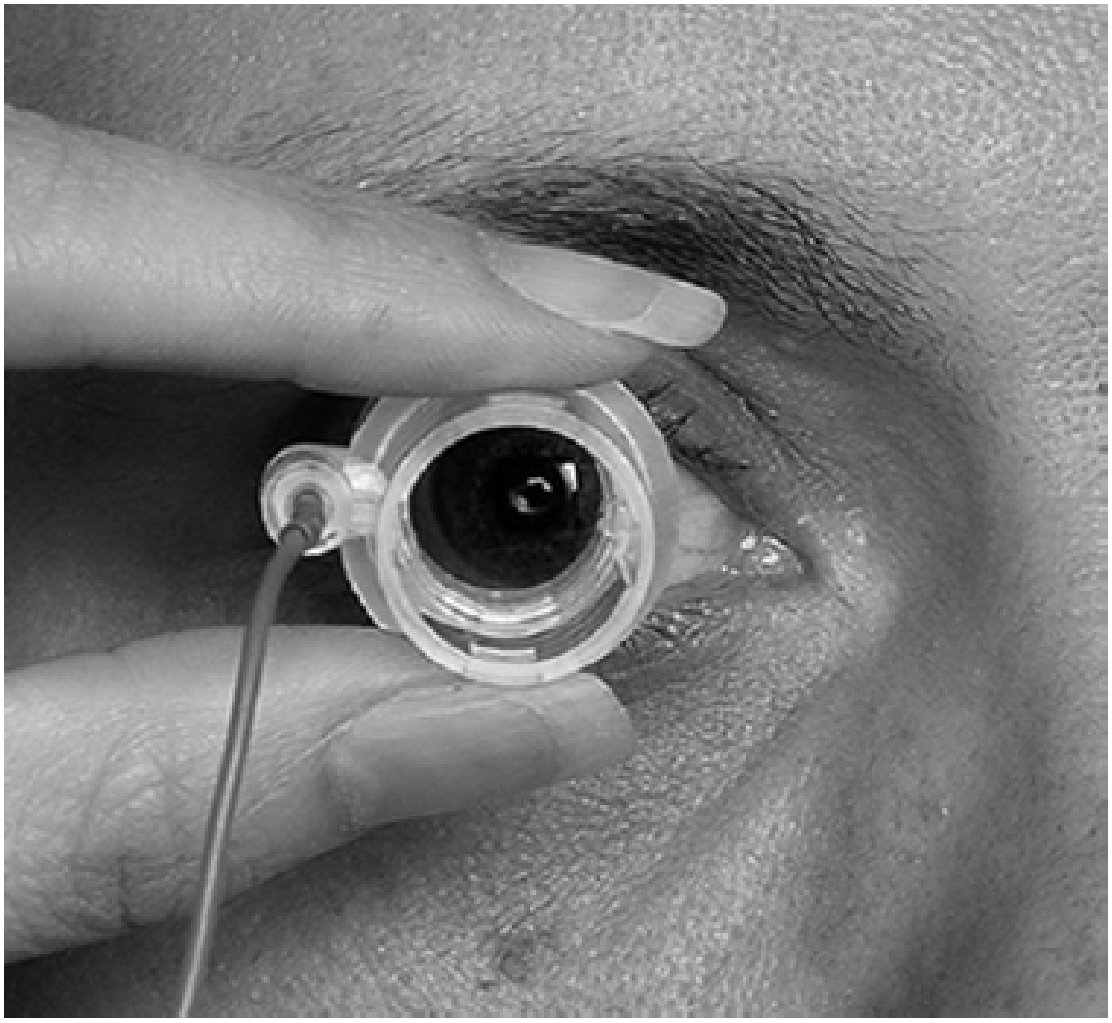

Figure 1.8. EyeGate® II Delivery System: A Transcleral Annular Iontophoresis Device.

Despite being dramatically less invasive than injectable or implantable dosage forms and offering much higher absorption and retention than most topical systems, the major limitation is that these systems require inconvenient doctor's office visits and the 6 month to 1 year sustained effect from implantable or injectable system is superior at this point. Future efforts are being pushed into developing sustained release dosage forms to be delivered by this method such as with the Visulex system™. This system draws the drug molecule and the counter ion into the tissue where it can precipitate and form a depot. While this technology is promising and exciting, this technology is still in early stage clinical trials (90).

OPHTHALMIC SPRAYS/MISTING DEVICES:

Versidoser ™ (Mystic Pharmaceuticals, Austin, TX, USA)

This platform device uses novel unit dose packaging that is incorporated into their multi-dose delivery device. The unit-doses can be formulated preservative-free and when used in the device they allow the patient to conveniently administer a 12-15 µL spray to the ocular surface. This device is equipped with a dose counter and ergonomic design to assist in patient compliance (91) . A pilot study published in 2010, investigated the use of a Nautilus 1.0 VersiDoser™ was used to administer 30 µL of the investigational drug MC-1101 to 11 patients with (dry) age-related macular degeneration, and to 20 normal patients aged 50 - 89 years old. The goal was to investigate MC-1101's ability to restore choroidal blood flow, which helps alleviate the progression of AMD at this stage. They found that the compound was well tolerated in both populations and choroidal blood flow

and velocity was increased in all patients that received the active compared to vehicle, however patients with AMD had a more variable response. The authors attributed this to the difficulty in assessing these parameters in this patient population due to the disruptive physiological state, and that further testing would need to be conducted to establish the role of MC-1101 in modulating choroidal blood flow (92).

Optimyst™ Nebulizer

The Optimyst™ ophthalmic nebulizer is a misting delivery device that contains a disposable medication reservoir that is intended to sterile filled. The solution is propelled through an ultrasonic or vibrating mesh actuator to form droplets in the 1 - 10 μm range, which can be seen in Figure 1.9. The aerosol generator is able to both, form the droplets as well as impart a forward inertia and allow them to propagate towards the eye. To administer the medication, the patient would hold the device in front of the eye and depress a button sending stream of droplets outward in plume in the eye.

Figure 1.9. Optimist Ophtalmic Nebulizer Prototype Device emitting a spray plume (Collins et at., 2007)

In 2007, a clinical study conducted by Collins et al. evaluated the effectiveness of a topical mist (6 μL) versus an instilled solution of 30 μL. They administered a topical mydriatic, Tropicamide ophthalmic solution 1.0%, and evaluated the change in pupil diameter over a 3 hour period. They observed the peak action in about thirty minutes for each case and there was no significant difference in average pupil diameter between that of the mist and that of the solution. Essentially, they found that despite delivering 1/5th of the volume of solution at the same concentration; they could produce identical clinical responses. The device used in the proof of concept testing was a prototype, and because the geometry of the mist plume beyond 2 cm was amorphous, the authors were unclear as to the exact amount of drug substance reached the surface of the eye(93). Nonetheless, the efficacy of the mydriatic mist was demonstrated to be within the suggested bioequivalence standards of the FDA, but larger more controlled studies would need to be conducted to make this conclusion.

***Modified Respimat*® (Boehringer Ingelheim)**

The modified Respimat® device generates a slow moving aqueous spray and was previous developed by Boehringer Ingelheim for use as an inhaler. It was recently been investigated for use in ophthalmology as topical delivery device. Diestelhorst et al. evaluated the microspray device in 20 total patients that had either glaucoma, dry-eye or were healthy volunteers. They found that the microspray device was superior to eye drops in terms safety, tolerability and handing (94). In another study investigating the bioavailability of fluorescein, researchers found the microspray to have a much higher bioavailability (95). The authors suggest the use of this device can simply topical therapy and could improve adherence.

This aqueous spray was originally developed as a combination product used in pulmonary drug delivery. The aerosol is generated by using a heavy duty spring to force a small metered quantity of liquid through various micro-channels within the device, called the uniblock. Within these channels the liquid is accelerated to form into high velocity jets which impinge each other upon exiting in order to break up the liquid into fine micro-droplets. In addition, these impinging jets also aid in reducing forward velocity, resulting in slow moving aerosol. The Respimat® had unique advantages over other aerosol devices in that the plume developed much slower than traditional pressurized metered dose inhalers. This feature also bodes well for use in topical ocular drug delivery as droplet size and impaction force will both contribute to patient comfort. Compared to other metered sprays the aqueous droplets produced by the Respimat® device are much smaller, with a mass median aerodynamic diameter of only 2 μm(96). While metered pump sprays, such as those used in nasal delivery, produce droplets that typically have an X_{50} of between 20 - 50 μm (97).

One of the unique advantages of using the Respimat® for pulmonary drug delivery was that because it generates a slow moving plume, it can reduce the amount of droplet that impact the back of the throat the patient. In addition there is a decreased reliance on timing the inhalation maneuver compared to pressurized metered dose inhalers. This improves dosing reproducibility and thus improving the overall delivery to the lungs. However, this slowly developing aerosol could turn out to be a downside for use in ophthalmology as the duration of the plume generation needs to be faster than a typical blink response. Otherwise, patients would need to hold their eyelids open manually. Also, special care needs to be taken so the aerosol is moving fast enough to impart inertial deposition but not so fast as to cause damage to the ocular surface or any discomfort to the patient. Furthermore, because the aerosol plume generated is the shape

of a cone, as the distance from the nozzle increases the larger width of the plume and the lower the concentration of the droplets becomes. This increased distance ultimately leads to low ocular deposition, which Newman et al. documented while assessing the miss-use and off target effects of the Respimat® when used for inhalation (98). To further optimize the use of this device for use in ocular drug delivery, modifications to the geometry of the plume would need to be made so that the majority of the dose would impact the ocular surface with a comfortable impact force and not deposit on the surrounding eyelids and face. Overall, with a few modifications this device shows promise as a useful tool for delivering small volumes of liquid to the surface of the eye.

Sprays/Vaporizers onto Closed Eyelids:

In addition to applying topical sprays or mists to the surface of the eye, scientists have also investigated applying these sprays to the eyelids of closed eyes. While the spray will not be directed onto the eye surface, the liquid can come into contact with margins of where the eyelids meet. The medicament can then wick into the precorneal film and disperse locally (99). Early investigations showed that using this technique can increase the residence time because the active can be retained at the interface of the eyelids and the tears (100). In addition, this method overcomes some of the coordination problems that are associated with eye drop instillation technique. This strategy has shown some benefit in reducing potent drug exposure in pediatrics(48). However, there is a large amount of controversy with these closed eye sprays, because the inherent variability of applying the medication in this form and the possibility of irritation on the surrounding tissue.

Tunable Aerosols: Drug Loaded Toroidal Vortices (Aerosol Vortex Rings)

New research out of the University of Texas at Austin is focused on utilizing the coherent geometry associated with toroidal vortices in order to deliver aerosolized medication to the surface of the eye. These 'smoke ring' vortices are well known for their reproducible structure and offer a unique opportunity for controlling aerosolized drugs over a distance suitable for treating the eye. Typical aerosols are emitted as turbulent jets, with little or no control of the plume after it is emitted. These turbulent plumes tend to spread out in an erratic, often unpredictable fashion. Theses vortices however, maintain their structure and are able deliver micron sized droplets to the eye surface in a tunable and precise manner. Figure 1.10 depicts a drug loaded toroidal vortex prior to impacting the ocular surface (101). Because the aerosols produced from this device can be generated independent of the actuation, the velocity of the droplets throughout the geometric space of the plume can be tightly controlled. This high level of control allows for tunable deposition, thus improving accuracy and precision (101). Furthermore, these droplets are moving at a rate fast enough to reach the eye before any blink reflex but because the droplets are so small they impact the surface with a minute impact force. This technology could have big advantages for improving bioavailability, reducing systemic effects and even improve patient ease of use for those that have difficulty maneuvering standard eye drops. A large number of drugs can be formulated into this device, but because the volume of delivery is small, drugs that require large doses could require multiple doses.

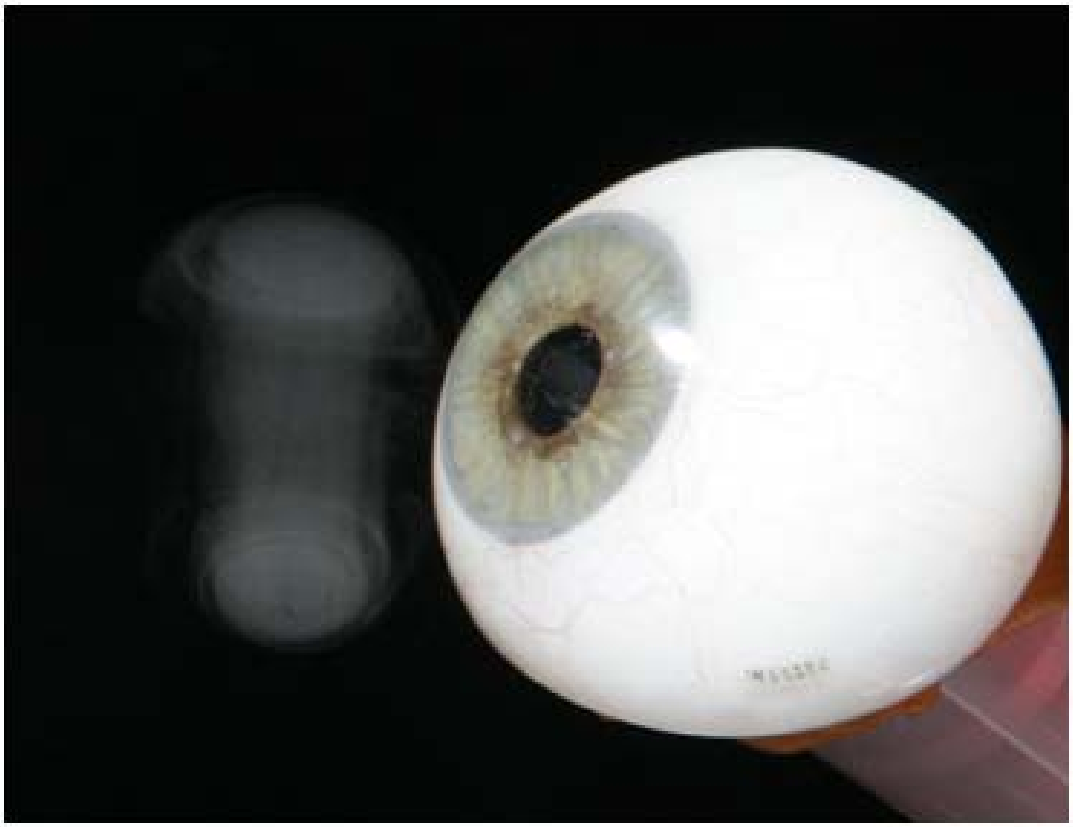

Figure 1.10. Image of a Toroidal Vortex Prior to Impaction on a Prosthetic Eye.

CONCLUSIONS:

While drug delivery to the eye is rife with challenges, many of the strategies and technologies highlighted herein offer a large number of solutions. As

advances are made in pharmacogenomics and patient centered drug design begins to take center stage, a similar approach must be taken from the dosage form and delivery device design aspect. With these advances in material sciences, microelectronics, smart polymers - safe, accurate and precision drug delivery is becoming more of reality. The future of medical care of the highest caliber hinges on contributions from multi-disciplinary scientific and engineering fields. All of which together can make great advances solving some of the multi-faceted and complex problems in modern medicine.

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Chapter 2. Research Objectives

Overall Objectives

As continued progress is made advancing and developing new therapeutic compounds to treat vision threatening disease, there is an equally important need to develop new and effective drug delivery technologies to ensure safety, efficacy and ultimately improved therapeutic outcomes. The primary objectives of this work were to investigate the use of aerosol loaded toroidal vortices for ocular drug delivery and to explore their potential roles in improving the standard of care.

Supportive Objectives

The first aim of this research was to characterize all of the physical characteristics of the toroid generating drug delivery device and to determine which features of the device are responsible for controlling dose output and performance.

The second aim of this research was to investigate different formulation strategies for incorporating poorly water soluble drugs into aqueous based vehicles, so that they may be optimized for use in the ocular drug delivery device.

SECTION 2: DEVICE MECHANICS AND PERFORMANCE

Chapter 3 : Precision Ocular Drug Delivery Via Aerosol Ring Vortices

ABSTRACT

Despite being the most prominent dosage form for topical ocular delivery, eye-drops have several well known drawbacks. In this work we introduce a novel method to accurately and comfortably deliver ophthalmic medications to the surface of the eye. We discovered that by aerosolizing a medicament and dispensing it in the form of a toroidal vortex, commonly known as a "smoke ring", some of the major drawbacks associated with topical drug delivery can be avoided. It was found that the dose delivered to the surface of the eye was directly proportional to the velocity and the size of the droplets emitted from the device. With a dilute solution, 0.05% fluorescein, doses could be reproducibly deposited by actuating the device at low velocity (~5 ng) and at high velocity (~18 ng). While with more concentrated solutions of 0.5% Fluorescein, between 20 and 160 ng could be deposited depending on selected actuation velocity. And with the highest concentration, 5% Fluorescein, 1.15 +/- 0.075 μg was deposited. In addition, the amount of drug deposited onto the eye surface was shown to be modulated by changing the chamber fill time. Precise toroidal vortex based aerosol delivery may facilitate optimized administration of medicines to the surface of the eye.

INTRODUCTION

The increasing prevalence of diseases associated with an aging patient population, especially in areas of ophthalmology and eye care have highlighted the need for

improved delivery systems. By far the most common method for delivering drugs the eye is the eye drop. While there is direct access to the eye, there are several formidable barriers preventing effective, efficient and/or convenient administration of the medicine. Indeed, eye drops are well known for having very low bioavailability; in some cases significantly less than 1% of drug is delivered at the desired ocular tissues (1, 2). This is well studied and is due to the multitude of physiological barriers that exist to protect the visual system, as well as the mismatch in the capacity of the eye to hold the instilled liquid contained within an eye drop. The pre-corneal film can only stabilize about 7 µl, yet a typical eye drop is on the order of 50 µl, and can range from 20 – 75 µl (3). This results in the majority of the eye drop immediately spilling over onto surrounding skin on the eyelids and/or it is rapidly cleared via the nasolacrimal drainage. Furthermore, the added liquid from the eye drop instigates further tear production promoting accelerated rinsing of the eye (4). Upon drainage the drug can be absorbed via the nasal cavity and enter the systemic circulation where potentially toxic off-target effects can occur, especially in pediatric populations (5-7).

Because of the highly protected nature of the eye, pharmaceutical scientists have proposed various formulation strategies to overcome these barriers. The two main principles for improving ocular bioavailability are increasing the permeation of the drug via permeation enhancers, transporter coupling or even modifying the drug's chemical structure to serve as a pro-drug with better pharmacokinetic properties (8, 9). Another approach is to increase the residence time of the drug with the relevant membranes (10). Increasing the residence time can be accomplished via many different methods such as: increasing the viscosity the formulation, incorporating mucoadhesive polymers, nano/microparticulates for mucin entanglement or even by using drug loaded ocular inserts, which reside semi-permanently in the ocular cavity.

Beyond the aforementioned strategies another method has been shown to improve the residence time in the pre-corneal film. It was as early as 1973 when Chrai et al. first published the correlation between instilled drop size and pre-corneal residence time (11). While studying the tear film formation and turnover he noted that as the volume of the drop instilled decreased, the longer it remained in the film. Since then many others have investigated this phenomenon (12-15). It has been proposed that by reducing the instillation volume, the reflex tearing and clearance mechanism is significantly reduced and subsequent improvements in bioavailability can be achieved (16, 17). Furthermore this reduced reflex tearing and drainage also reduces or eliminates unwanted systemic effects by reducing the amount of drug that enters the nasolacrimal system. While reducing drop volume is a promising strategy, there has been technical difficulty in developing systems in with patients can safely self administer micro sized volumes accurately either because of the technical complexity, variability of the spray plume or because risks associated with fine tipped droppers (18, 19).

One of the challenges with delivering small volumes is in the limit of the droplet size. Because of the surface tension in water it is difficult to form fine droplets, so typically large amounts of energy are required to overcome surface tension and aerosols are produced with high velocities and /or amorphous plumes that are difficult to control.

In this study we investigate a novel drug delivery device that is capable of delivering very small volumes via aerosol droplets for deposition onto the ocular surface. This device generates micron-sized droplets and forms them into a highly structured toroidal vortex (i.e. smoke rings) in order to precisely and reproducibly administer a drug to the ocular surface (as depicted in Figure 3.1). Unlike other sprays or mists which are difficult to direct due to the variability of the plume geometry and speed, these smoke rings hold together enabling additional control of the deposition. We investigate herein

the main device features that influence the device performance in regard to in-vitro drug deposition in hopes to fine tune the delivery for future pre-clinical testing.

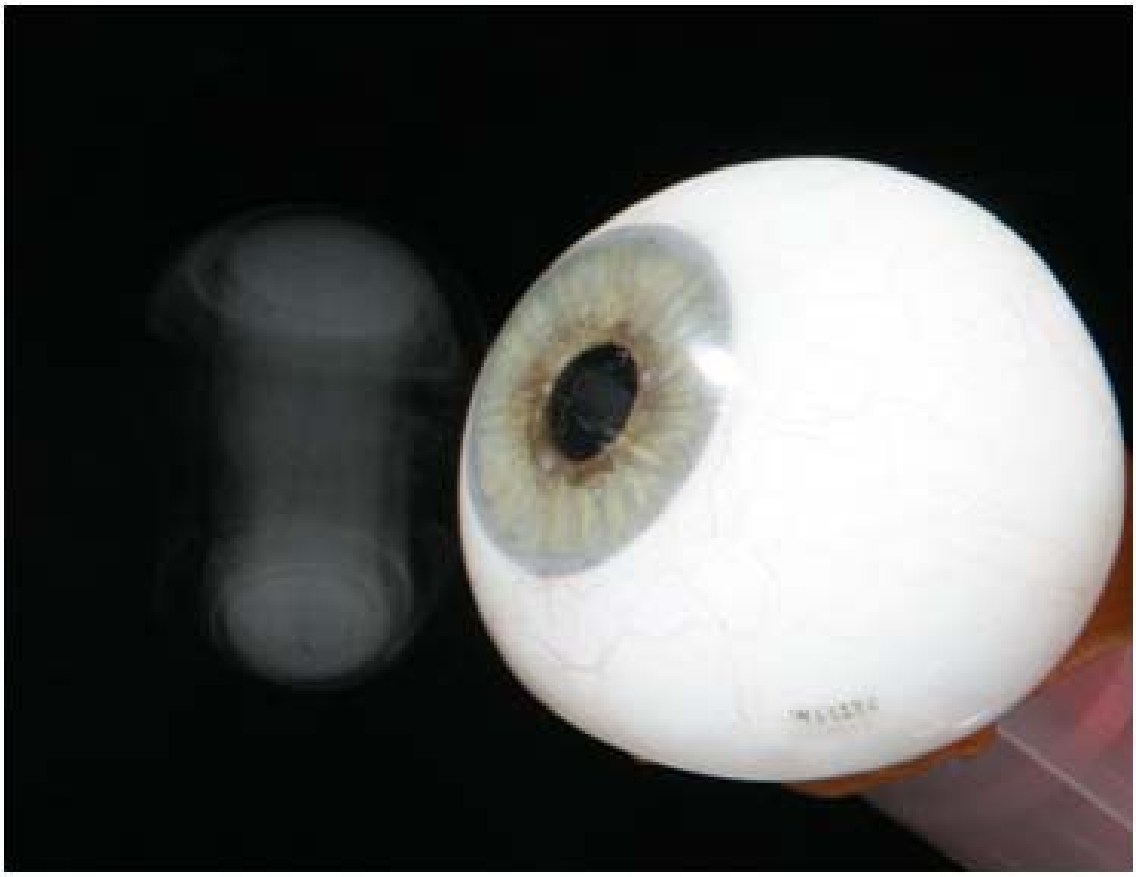

Figure 3.1. Image of a Drug Loaded Toroidal Vortex prior to impacting the surface of a prosthetic eye (Smyth and Herpin, 2015)

MATERIALS & METHODS

Generation of Fluorescein Loaded Toroidal Vortices.

The formation of the toroidal vortex vehicles were separated into two steps, (1) the generation of the aerosol and (2) the actuation of device. To form the aerosols we first prepared three solution formulations using sodium fluorescein (Sigma-Aldrich, USA) as the tag/model drug dissolved in isotonic phosphate buffered saline at pH 7.4 (Sigma-Aldrich,USA). These formulations were selected in order to cover a 100x change in concentration, from 0.05% to 5%, which represents a large spectrum of potential starting solution concentrations. The formulations were loaded into the sample cup of the Omron MicroAir® Nebulizer model: NE-U22, and the nebulizer was mounted onto the toroidal vortex device actuator. To control the aerosol chamber fill time, a stopwatch was synchronized with the manual activation of the aerosol generator. Upon filling the aerosol chamber with predetermined amount of aerosol the device was actuated with a predetermined force.

To actuate the device a calibrated customized pendulum equipped with a precision protractor for measuring set heights was used to vary the actuation force. The actuator device membrane was positioned in-line with the pendulum at the 90º, allowing for an accurate horizontal impaction force to be delivered the membrane and initiate the

toroid formation (see Figure 3.2**)**. When the membrane is actuated using the pendulum, the forward displacement generates an instantaneous pressure increase within the chamber and induces flow into the gas in which the aerosol droplets are suspended. This results in the aerosol exiting through the device orifice. The initial fluid flow at the edge of the orifice creates an area of low pressure (i.e. a vortex) along the pathway of the orifice. This results in the formation of a circular vortex also known as a toroidal vortex or more commonly known as a 'smoke ring'. The emitted vortex can then be characterized in terms of velocity and droplet size distribution, and in-vitro deposition.

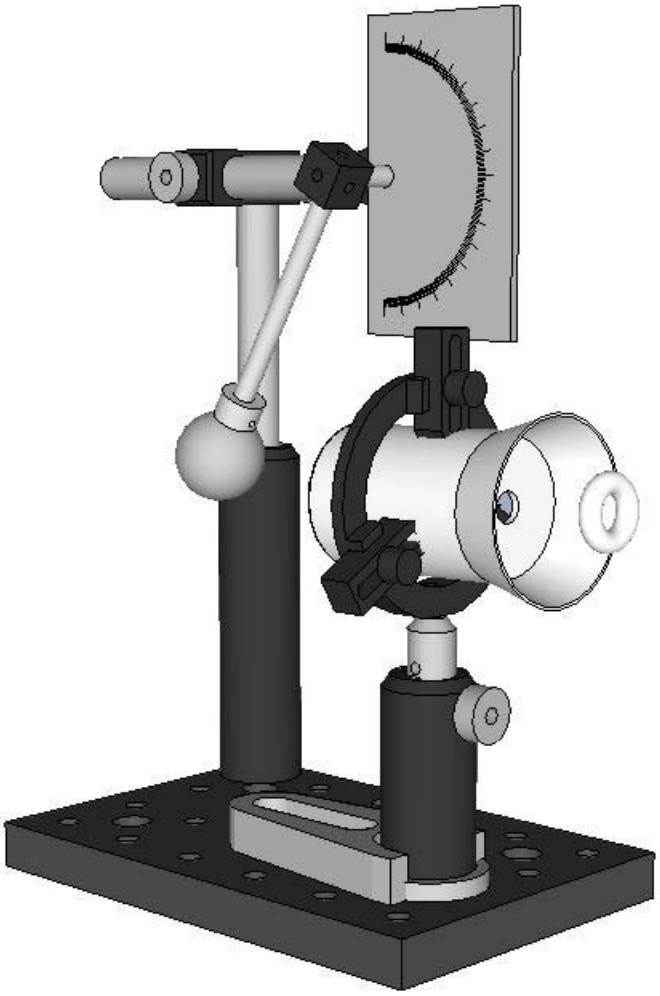

Figure 3.2. Ocular Drug Delivery Device attached to a Calibrated Actuator(20).

Vortex Translational Velocity

To investigate the relationship between impaction force and toroidal vortex bulk velocity, we utilized an analogous method to that of Hochrainer et al., known as the "dark field technique" (21). For this method, the device was placed in a dark box and aligned with a spatial calibration target. The pathway for the emitting vortices was illuminated by a laser sheet and then the device was actuated at various relative forces using the pendulum. As the aerosol is emitted the droplets are illuminated by the laser sheet and a high speed camera was used to capture the video recordings at 200 frames per second. The digital images were processed using front edge of the vortex to track the velocity of the propagating vortex. The velocity measurements were done using Kinovea Video Analysis software.

Deposition Studies: Inertial Impaction Simulations

The deposition of entrained droplets onto a surface is a very important parameter for the performance of the device as it will indicate the dose delivered to the eye. The deposition mass is the amount of drug that leaves the toroidal vortex upon impaction on the surface of the eye. In initial testing a prosthetic eye was used to simulate geometry of the eye to achieve spatially similar impaction. For screening studies we utilized next-generation inertial impactor (MSP Corp, MN) impaction plates as the substrate. These impaction plates are designed for inertially collecting aerosols during analogous testing. The device outlet was fixed at a distance of 3 cm from the plate. The aerosol chamber was filled for predetermined and varied amounts of time with each formulation. The

device was actuated at a range of actuation forces in order to assess the effect of chamber fill time and actuation force on aerosol deposition.

Quantification of droplet deposition

The impaction plates/deposition substrates were rinsed with adequate diluent and assayed for mass deposition by fluorescence spectroscopy with a 460 nm excitation wavelength and a 515 nm emission wavelength, using the Tecan M200 Microplate Reader.

Particle/Droplet Size Distributions

The droplet size and distribution of the aerosols produced from the device was measured using a Sympatec Helos laser diffraction apparatus (Clausthal-Zellerfeld, Germany). The aerosol was emitted directly into the laser beam at ambient conditions. The laser diffraction pattern was collected using the R3 lens (f= 100mm) and the calculations were done using Fraunhofer theory with the Sympatec Windox 5 software.

RESULTS AND DISCUSSION

Formation of Toroidal Vortex Rings

In order to generate drug loaded toroidal vortices two main actions were necessary. Firstly the device chamber needs to be filled with an aerosolized formulation and secondly, a force applied to actuate the device. In our case we used a vibrating mesh

nebulizer as source of reproducible droplets that are in the size range desired. The Omron MicroAir NE-U22 device utilizes a horn transducer that oscillates at 180 kHZ. This horn is adjacent to a micro-perforated mesh with approximately 6,000 holes (22). The solution sample is pumped from a reservoir through the mesh which is directly in contact with allowing for small sample volumes to be utilized. The aerosol produced from the generator fills the loading chamber just prior to device actuation. Figure 3.3 is a close up graphic showing the device a emitting toroidal vortex from the orifice which is directly adjacent to chamber from whence it is dispensed. This allows for rapid chamber filling and subsequent ejection. Finally, the large rim of the device is the eye-piece. This is used to correctly position and space the device relative to the eye, as well as to assist in holding the lower eyelid in place to prevent blinking.

Impaction on the Ocular Surface:

In Figure 3.1, the relative size of the toroidal vortex to ocular surface can be shown. Keeping in mind that the majority of sclera will be covered by the upper and lower eye-lids, the cornea however is the portion directly covering the colored iris and is predominantly exposed. The geometry of these toroidal vortices was designed so that the impaction portion would be directly centered with that of the cornea. In the case of administering this dosage form, the device is aligned via the surrounding eye-cup depicted in Figure 3.3 , where the eye- cup is placed onto the ocular orbital comparable to looking into a binocular eye-piece. In addition the lower eye-lid may need to be held down briefly in order to maximize the available surface. This is comparable to what is done with eye-drop applications however in this case the head will not be required to be tilted backward as the dosage is an aerosol which disperses into the existing tear film.

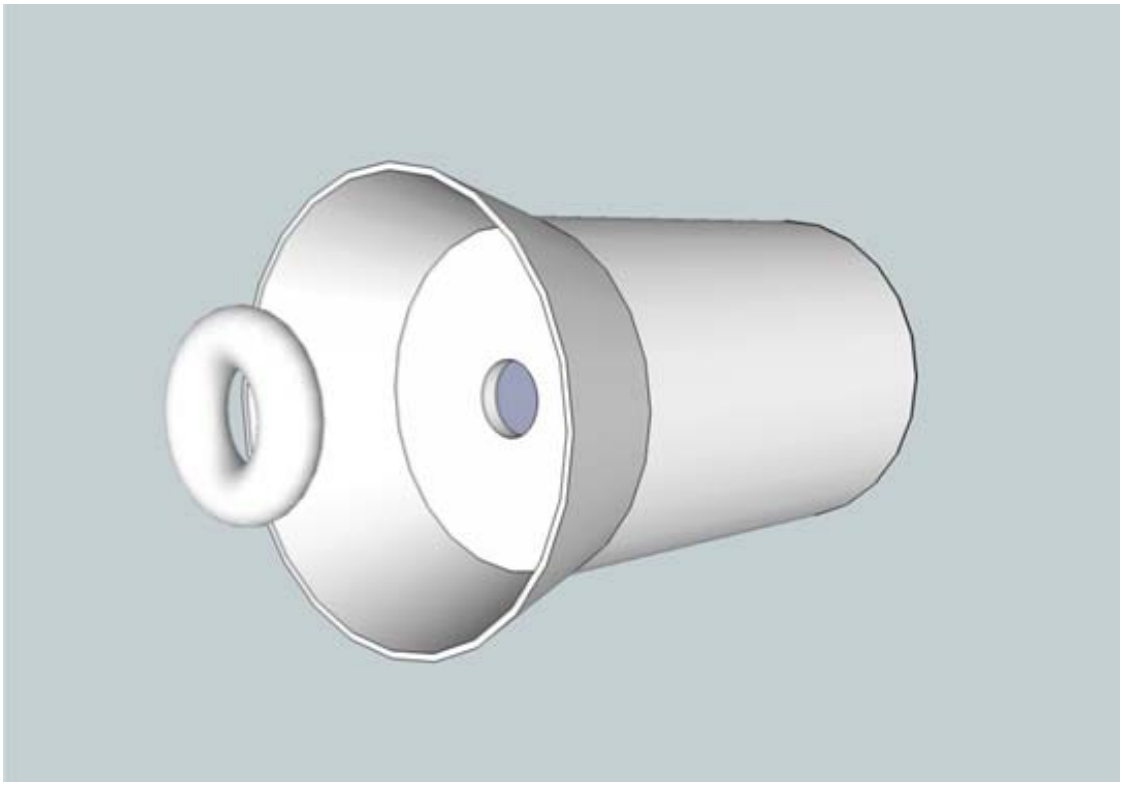

Figure 3.3. Illustrative depiction of the ocular drug delivery device emitting an aerosol loaded toroidal vortex

In-Vitro Deposition Analysis

In preliminary testing, an ophthalmic prosthesis was used as a substrate for deposition because of the analogous geometry. The extraction of the deposited material from the prosthetic introduced sources of error attributed to extraction efficiency. Therefore, to assess the in-vitro deposition of the output from the device we utilized Next Generation Impactor testing plates. These plates are designed for the capture of impinging aerosol streams used in the assessment of aerodynamic diameter for aerosolized dosage forms. It has been suggested that the surface roughness may play a role in deposition ballistics (23). These plates are manufactured to a have specific surface roughness as per USP compendia to be between 0.5 and 2 μm. In addition, these plates are easily grounded to reduce any effect of electrostatics in that could be generated with electrically insulating components during the course of this assay (24).

To conduct the deposition analysis, the device was placed a distance of 3cm from the plate. This distance was selected as it provides adequate time for the toroidal vortex to fully form and propagate towards the eye. It is also no so far that the dynamics of the toroid are disrupted due to turbulence. The device was filled with aerosol and actuated to produce toroids moving at different velocities. The range of velocities screened for deposition was such that at the lowest velocity to form a vortex by this mechanism all the way to the top end where the toroidal vortex breaks down into a turbulent jet. Others have been able to produce stable vortices with higher velocities via different actuation mechanisms, for evaluation of different phenomena, for our purposes these ranges suffice (25).

The main purpose of this experiment is to understand the relationship between toroid velocity and deposition onto a substrate. Aerosols have 4 main mechanisms for

depositing: inertial impaction, sedimentation, intersection and diffusion (26-28). In addition there are several other mechanisms for deposition such as thermophoresis and electrostatics which at this point these mechanisms are beyond the scope of this article. We hypothesized that the deposition of the droplets would be proportional to the velocity, as the velocity component of momentum in the equation, momentum = mass x velocity, is proportional to the mass and thus the inertial of the droplets. In addition, in these experiments we were not altering the size/mass of the droplets at each velocity, so any possible changes in deposition would likely be correlated with velocity. As can be seen in Figure 3.4, with the 0.05% fluorescein solution, as the velocity increased, the amount of fluorescein deposited also increased. At the lowest velocity, about 5 ng was deposited, while at the highest velocity between 12 and 18 ng was deposited. Likewise intermediate velocities showed intermediate deposition. This is a useful finding as it reveals that with a constant chamber fill time the amount of aerosol that is emitted should be constant, yet more of the emitted aerosol is impacted onto the surface. This indicates a much higher deposition efficiency at higher velocities. This same effect was more exaggerated at the higher drug loading concentration of 0.5% (Figure 3.5). At this concentration the minimum deposition was approximately 20 ng and the maximum was about 160 ng. This shows a nearly 10X increase in deposition just based off the toroid velocity. In addition, this shows that with just 2 different formulations a nearly continuous range of deposited quantities can be achieved by altering the device actuation force.

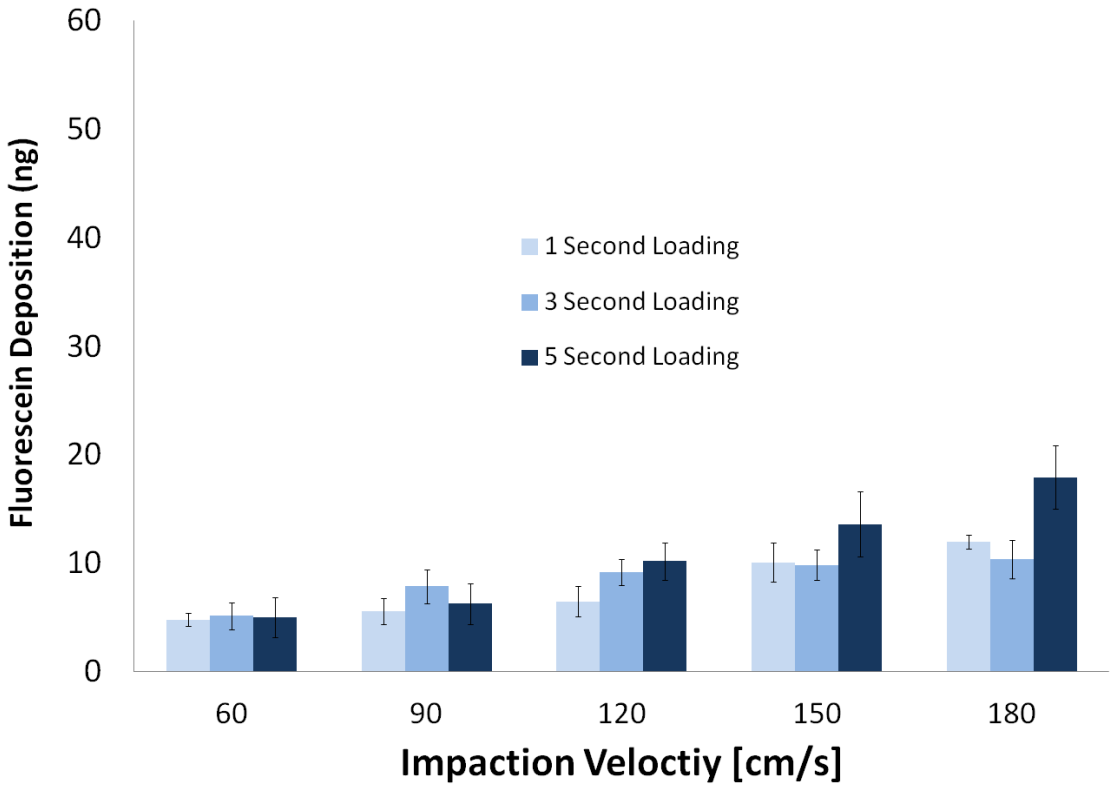

Figure 3.4. In-Vitro Deposition Analysis. 0.05% Fluorescein. Fluorescein Deposition as a Function of Toroid Impaction Velocity and Chamber Fill Time.

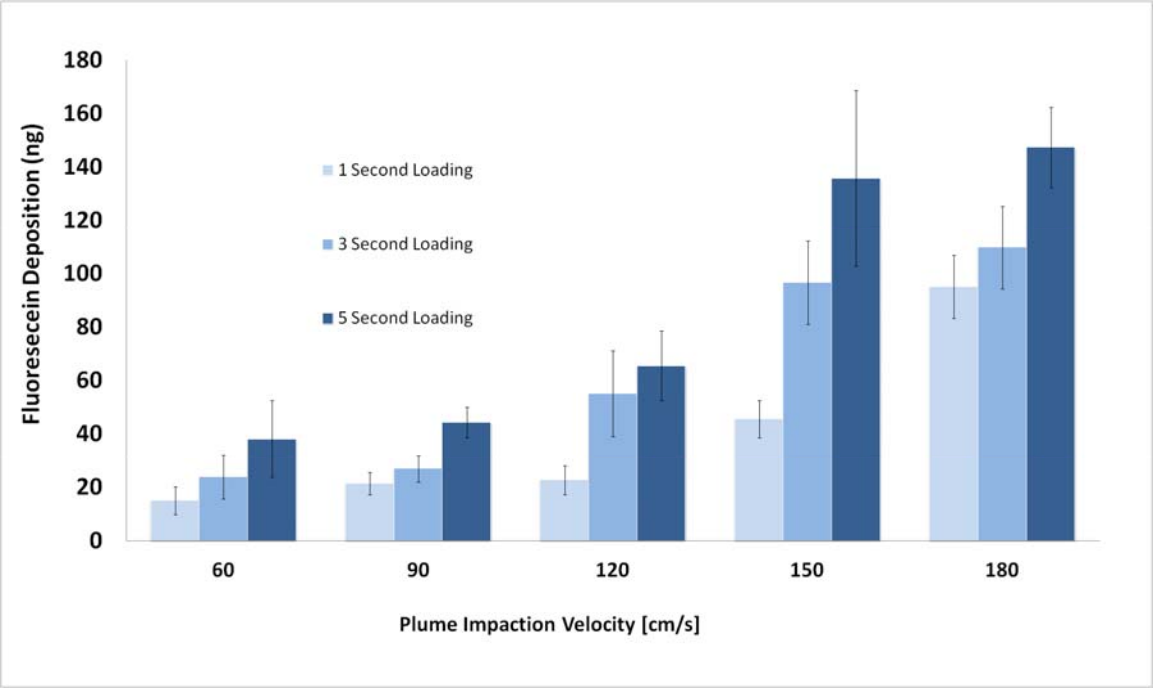

Figure 3.5. In-Vitro Deposition Analysis. 0.5% Fluorescein. Fluorescein Deposition as a Function of Toroid Impaction Velocity and Chamber Fill Time.

Chamber Fill Time:

Another aspect of this study was to investigate the effect of chamber fill time on the amount of drug deposited. We hypothesized that as the chamber fill time increased, the number of droplets in each toroid would also increase therefore increase the available number of droplets to be deposited onto the surface via impaction. For the 0.5% solution at each toroid velocity, as the chamber fill time increased the deposition increased Figure 3.6. However, in the case of the more dilute formulation, 0.05% fluorescein, the deposition did not increase as drastically over the range of chamber fill times Figure 3.7. This is due to the diminished amount of drug being added per quantity of time in dilute solutions. A 1, 3 and 5 sec fill time only allows for a small quantity of drug to be added at a fixed nebulization rate. At a fixed nebulization rate droplets are being formed continuously and each new droplet only slightly contributes to an increase in deposited drug mass. On the other hand, while each second with a concentrated solution nebulization rate contributes much more drug by droplet volume/density (i.e. higher drug payload per droplet/time).

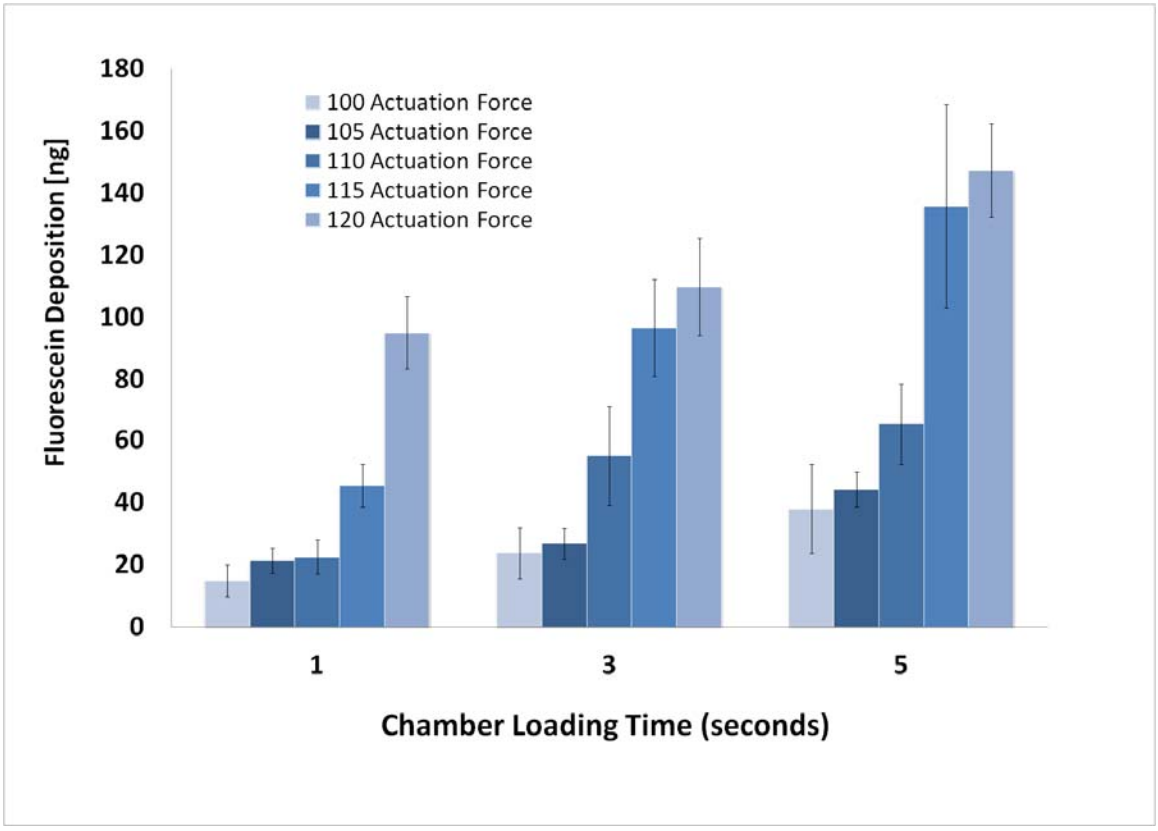

Figure 3.6. 0.5 % Fluorescein Deposition as a Function of Chamber Fill Time at Different Actuation Forces.

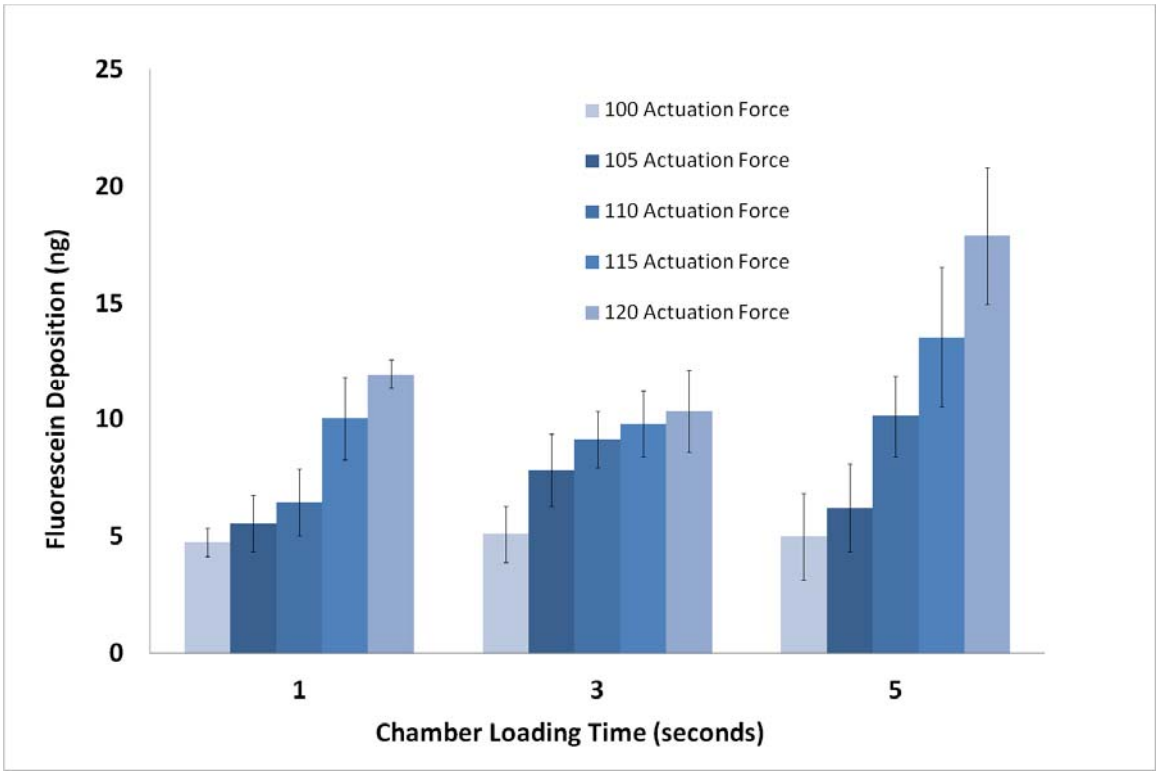

Figure 3.7. 0. 05 % Fluorescein Deposition as a Function of Chamber Fill Time at Different Actuation Forces.

Dose Uniformity under Upper Loading Conditions

To determine the amount of drug deposition that can be achieved when using a more concentrated 5% fluorescein sodium solution, the device was loaded for 5 seconds and actuated at the highest velocity. With this concentration, the device was able to deposit 1.15 μg +/- 0.075 μg. While this is a relatively small mass of drug overall, it will be instilled into the very small volume of the tear film. This could theoretically result in a very high local concentration and contribute strongly to establishing a large diffusion gradient. Furthermore, because the eye is such a small organ this quantity of mass is on the order of what is need to achieve adequate EC50s for many commonly used ophthalmic medications, assuming the drug is sufficiently permeable. Our lab intends to focus future investigations in exploring the *ex vivo/in vivo* pharmacokinetics of these micro-volume instillations.

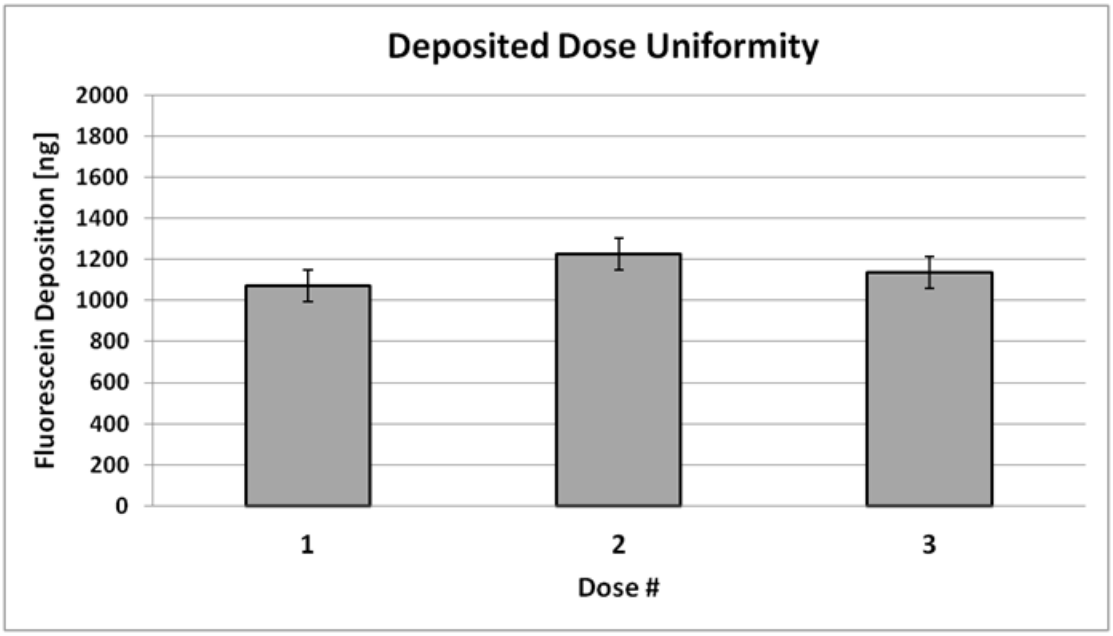

Figure 3.8. Deposited Dose Uniformity from 5% Fluorescein, with a 5 Second Chamber Fill Time.

Particle/Droplet Size Distributions (PSD)

The production of droplets for use in this device is done achieved with a vibrating mesh nebulizer. While many different types of aqueous aerosol generators may be used to provide aerosols with adequate characteristics, he vibrating mesh nebulizer here (OMRON MicroAir) produces uniform and reproducible droplets which tend to be robust in size for differing formulations (29, 30). Use of these nebulizers in the eye is new area of research, however several others have investigated the formulation affects on droplets size and output rate in regards to pulmonary drug delivery (31-34). Here in these studies we are primarily concerned with large changes in size that could affect the settling time/chamber fill time ratio. In that when droplets become too large they tend to settle more rapidly, as described by Stokes' Law, and it is crucial that droplets are suspended in the chamber long enough to be ejected in the toroidal vortex. On the other hand, the droplets cannot be so small that they are not easily ejected from the vortex upon impaction onto a surface.

Altering the concentration of the drug in the solution will have an effect on the physical chemical properties of the solution and its interaction with the vibrating mesh could alter the size of the droplets, we analyzed the droplets size distribution for each of the formulations. As can be seen in Figure 3.9, the sizes are nearly identical for each of more dilute solutions (0.05% and 0.5%) with the mode being right after about 5.5 μm. The main difference in PSD between the formulations is that the dilute solutions, 0.05 & 0.5 % have a more broad distribution with larger tail on the large size end, and the highly concentrated, 5% solution, had a much more narrow PSD spread. In Table 3-1 the data

shows the 0.05% and 0.5% solutions to have nearly identical cumulative makers, with X50 of ~ 5.8 μm, an X_{10} of ~1.4 μm, an X_{90} of ~14.5 μm, and with a volume mean diameter (VMD) of about 7.1 μm. As for the most concentrated solution, 5% Fluorescein, the particles size distribution was shifted toward the smaller size, largely due to the reduction of the tail. This decrease in droplet size could be attributed to a change in surface tension, and be explained by Tate's Law (32, 33, 35). Furthermore, because the solution is much more concentrated, other contributions from ionic strength or changes in micro-rheology cannot be ruled out. Nonetheless, all the droplets produced in with the vibrating mesh nebulizer were small enough to form stable aerosol clouds by visible inspection and none of droplets were so large as to settle before being ejected from device.

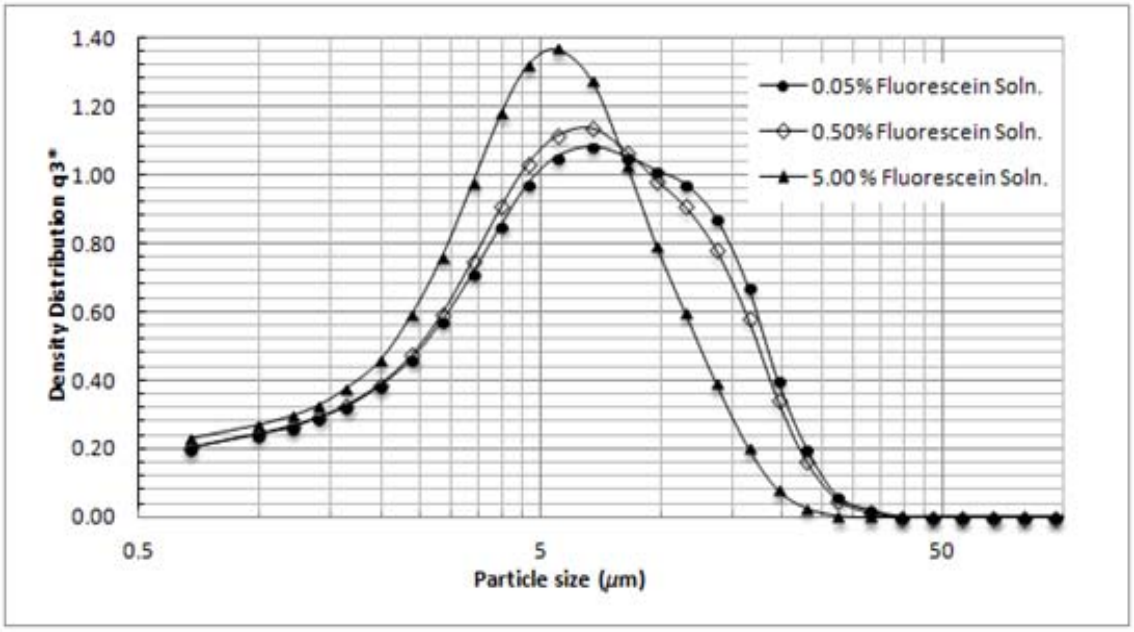

Figure 3.9. Particle/Droplet Size Distributions of the Aerosols Produced from 3 different Fluorescein Formulations.

Table 3-1. Fluorescein Formulations with Particle Size Distribution Data Measured by Laser Diffraction

Formulation	X_{10} (µm)	X_{50} (µm)	X_{90} (µm)	VMD (µm)
0.05% Fluor.	1.39 (0.01)	5.86 (0.14)	14.65 (0.42)	7.14 (0.21)
0.50% Fluor.	1.38 (0.4)	5.74 (0.12)	14.42 (0.35)	7.01 (0.18)
5.00% Fluor	1.27 (0.01)	4.73 (0.03)	10.53 (0.29)	5.47 (0.08)

Currently aerosol generating devices are designed in order to produce droplets in the 1- 5 μm range so that they can navigate the pulmonary system and be deposited into the deep airways. If they are too large they deposit in the mouth and throat and if they are too small they are exhaled. A similar challenge exists in designing aerosol for topical impaction. The droplets need to be small enough to be entrained into fluid vortices but also need to be large enough to carry enough inertia for deposition and to deliver an adequate payload. However, the droplets cannot be so large they rapidly settle or unable to be entrained in the fluid flows. The vibrating mesh technology is well suited for this application because the size of the laser drilled holes in the mesh are highly correlated with droplet size and can be easily altered for different formulations. As can be seen in data present in this article the droplet size and distributions can be altered slightly, but for the most part the range is adequate for our ophthalmic purposes.

CONCLUSION

In this study we were able to successfully produce drug loaded toroidal vortices containing a model drug fluorescein, and characterize several of the key features of the device and how they relate to in-vitro performance. Furthermore we established the relationship between drug deposition and vortex velocity in hopes to determine the most adequate ejection velocity for future drug delivery attempts. Many of the control features such as chamber fill time and ejection velocity appear to offer a direct means for tuning drug delivery doses from the device. This should allow for a highly tunable delivery mechanism that could be altered to accommodate a large number of medicinal agents at a

wide range of concentrations. While in-vitro testing reveals the device is capable of precision delivery, ex-vivo or in-vivo testing would need to be done in order confirm if the delivery to the ocular surface is in fact just as precise. Furthermore, in-vivo pharmacokinetic analysis would need to be done in order to establish if this device offers any additional enhancement in bioavailability as predicted by the theory and demonstrated by other methods.

ACKNOWLEDGEMENTS:

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Chapter 4 : Characterization of Drug Loaded Toroidal Vortices Intended for Ocular Drug Delivery

ABSTRACT

For the last several decades the predominant method for delivering medicine to the surface of the eye has been the standard multi-use eye drop. While being the most popular, this method has significant limitations. Recently an effort has been made to explore the use of a toroidal vortex or "smoke ring" aerosol delivery system that may help overcome these limitations and enable delivery specific amounts of formulation and drug to the ocular surface. Previous studies have shown that reproducible quantities of medication can be deposited *in vitro*; however, the physical characteristics of the aerosol output in regards to device performance and patient comfort have yet to be established. Here, we investigate the mechanics and the nature of these vortices, such as translational and rotational velocity, regional droplet size distributions and relative impact force in order to optimize the performance and evaluate their clinical relevance as a drug delivery vehicle for future investigations. Maximal droplet velocity at various actuation forces was determined, and they were all less than 6 m/s even at the highest actuation forces. Moreover, plume impaction forces were determined and were all less than about 4.5 micro-Newtons. Collectively, these studies showed that the physical and mechanical properties of the emitted drug loaded vortices would be suitable for ocular administration.

INTRODUCTION

The increasing prevalence in the age related disease is especially notable in ophthalmology. It is estimated that between 1 - 4% of all patients over 45 years of age will be diagnosed with glaucoma (1). And between 6 - 22% patients over the age of 70

years will be afflicted with age-related macular degeneration (2). These ocular diseases are the two leading causes of blindness in the United States, which collectively, major visual disorders cost $35.4 billion annually, and is expected to further increase with an aging patient demographic (3).

Despite new ophthalmic drug candidates to treat glaucoma and retinal disease, large barriers to their effective delivery to the target tissues still remain. This is due to the highly protected nature of the ocular/visual system and its crucial role in survival throughout the evolutionary process. Beyond the physical structures in the eye such as the cornea, sclera, iris, and cilliary body that can obstruct the diffusion of drugs from the front of the eye, there is also blood-retina barrier, and the blood-aqueous barrier. These barriers limit the entry of drugs from the blood stream so that many systemically circulating medications do not achieve adequate concentrations in the ocular tissues (4). The eye is also guarded with a reflex blinking and flushing system from the anterior. This system drastically reduces the amount of time a drug has to diffuse across the barriers of the front of the eye. Conventional ophthalmic drug delivery systems such as viscous solutions, suspensions, gels and ointments attempt to increase the residence time and thus the exposure, to improve absorption. However, due to the difficulties in administration and increased blurring of the vision, patient compliance becomes an issue. Other, more non-conventional forms such as ocular inserts or drug eluting punctal plugs have also been investigated and used for the purpose of decreasing dosing frequency and improving bioavailability (5). While these have shown to be promising they have limitations in that they can be inadvertently ejected from the eye which often requires a doctor's visit to reinstall the device. In addition, some patients complain of discomfort in wearing them, so at this point they have not been able to provide an overall solution yet.

Recently pharmaceutical scientists have investigated the potential use of ophthalmic aerosols to deliver medications to the eye. In a clinical trial assessing mean maximal pupil dilations in 100 patients, Collins et al. found no significant difference between eye drops and an ophthalmic aerosol administered topically (6). Moreover, others have previously shown improved drug bioavailability from smaller volume instillations (7-11). In addition, the use of small volume instillations can significantly reduce the overall drug exposure to the patient and potentially eliminate or reduce some off-target effects, which is especially important in pediatrics (12-15).

Our laboratory has recently developed a method to deliver medications to the surface of the eye via aerosols which are formed into toroidal vortices (e.g 'smoke rings') that enabled accurate and controllable delivery of small volumes (16). Because this method of delivery is significantly different from other aerosol or small volume approaches tested previously, we investigated here, several different analytical techniques for the characterization of this aerosol delivery system. This delivery technology produces micron sized droplets containing dissolved drug, moving at variable velocities. Therefore advanced analytical methods needed to be adopted to characterize this dynamic system.

The objective of this study was to characterize all of the clinically relevant physical performance features of this delivery device, in order to determine if the device performs in a safe comfortable and reliable manner for future clinical investigations.

MATERIALS AND METHODS:

Materials:

The Omron MicroAir NE22 vibrating mesh nebulizer was graciously donated by Omron Corp. EZ-Breathe Atomizer (Nephron Pharmaceuticals, Orlando, FL) was purchased OTC. All formulations utilized isotonic phosphate buffered saline, PBS (Sigma), or PBS and Fluorescein Sodium which was purchases from Spectrum Chemical MFG Corp., USA. And the ocular toroidal vortex generator was custom fabricated in house.

Methods:

Device Actuation for the Generation of Toroidal Vortices

Generating toroidal vortices from this ocular drug delivery device is based off two key major actions/steps, (1) the aerosol generation and (2) the ejection of the droplets. Aerosols were loaded into the chamber portion of the device via two different vibrating mesh aerosol generators, the Omron MicroAir NE-U22 and the EZ-Breath Atomizer. Once the chamber is adequately filled with the desired amount of aerosol, a 12-volt push-pull type solenoid (Adafruit,USA) is activated in order to impart a force wave onto the actuating membrane. And a variable voltage controller was used to alter to the actuation energy supplied to the membrane actuator and thus alter the exit velocity of the toroidal vortices. This device actuation mechanism is illustrated in Figure 4.1.

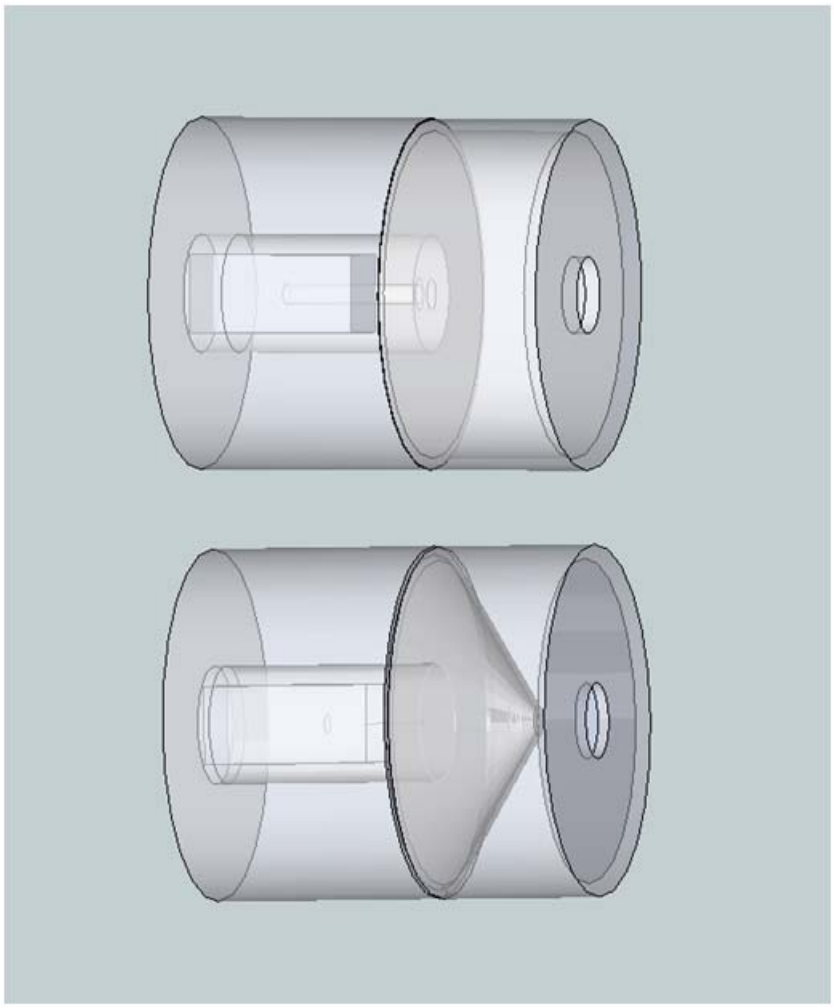

Figure 4.1. Illustration of the Actuation of the Ocular Delivery Device.

Determination of the Maximal Droplet Velocity within the Toroidal Vortex

Stereo Particle Image Velocimetry:

High-speed stereoscopic particle image velocimetry (PIV) was utilized to measure the velocity field in a plane cutting through a propagating toroidal vortex with nebulized droplets entrained within. We generated a laser beam with a high-repetition rate Nd:YLF laser (Coherent Evolution 90) that was then formed into a thin sheet with bench top optics. This laser sheet was used to illuminate the droplets within the toroidal vortex at a frequency of 5 kHz to obtain a velocity field every 200 μs. This laser sheet is 25 mm wide and aligned with the device orifice (nozzle) to allow for tracking the velocity as it is generated to a distance of 25 mm. The spatial orientation can be seen in Figure 4.2. Furthermore, two high-speed Photron APX cameras fitted equipped with Nikkor 105 mm lenses with an f/5.6 aperture were fixed at an angle focused toward the propagation path of the toroids in order to capture any out-of-plane motion.

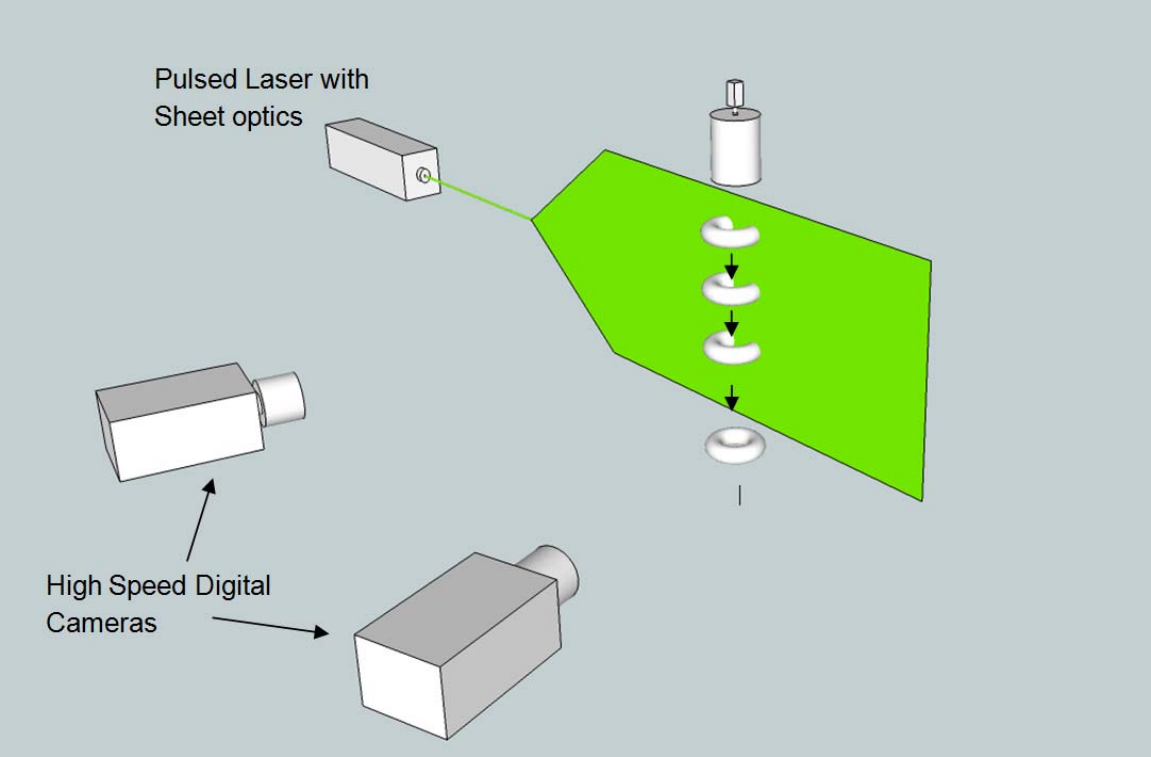

Figure 4.2. Schematic of the Experimental set-up of Stereoscopic Particle Image Velocimetry

The investigational space was calibrated with a dot target of known dimensions so that a mapping function between pixel location on camera and physical space could be established. The cameras were synchronized with the laser to capture light scattered off of the droplets at 5 kHz at a resolution of 512^2 x 512^2. For PIV processing the images were divided into 16^2 x 16^2 interrogation windows with 50% overlap. The particle images in subsequent interrogation windows over time are cross-correlated to obtain the particle displacement. The velocity vector is obtained from the particle displacement and the time delay between two subsequent laser pulses (200 μs). We used the DaVis 8.2 Software package by LaVision to perform PIV processing at a spatial resolution of 0.055 mm/pix, which leads to a measured velocity vector every 0.45 mm allowing for well resolved velocity fields throughout the internal dimensions of the vortex.

Toroidal Vortex Translational Velocity

In order to determine the velocity at which the entire toroid propagates a different approach was taken. The high-speed image sequences allow for temporally resolved measurement of the velocity by mapping them to physical space via the pinhole calibration previously mentioned and employing Matlab in conjuction with its image processing toolbox. The velocity of the toroidal vortex is determined based on tracking the front edge of the toroid over time, this comparable to what others have done using the "Dark Field Technique" to characterize metered dose inhalers (17). Except in this case the front edge detection was automated and based off an intensity threshold. And the

velocity evolution was obtained by a sliding linear curve fit to the change in position over time.

Droplet Size Analysis

The aerosols produced from this device were characterized for particle/droplet size distribution (PSD) and optical concentration (Copt) by laser diffraction (Sympatec-Helos) using the Fraunhofer (HRLD) and Mie Theory calculation methods and data was collected with used of an optical trigger. The modular dispersing units were removed and the instrument was set-up in the 'open-bench' mode where the aerosol can be transmitted from the device directly into the beam path for analysis. The instrument was set to collect PSD/Copt data at a 1 ms intervals throughout the duration of the toroid propagation. A schematic how the vortices where analyzed is illustrated in Figure 4.3.

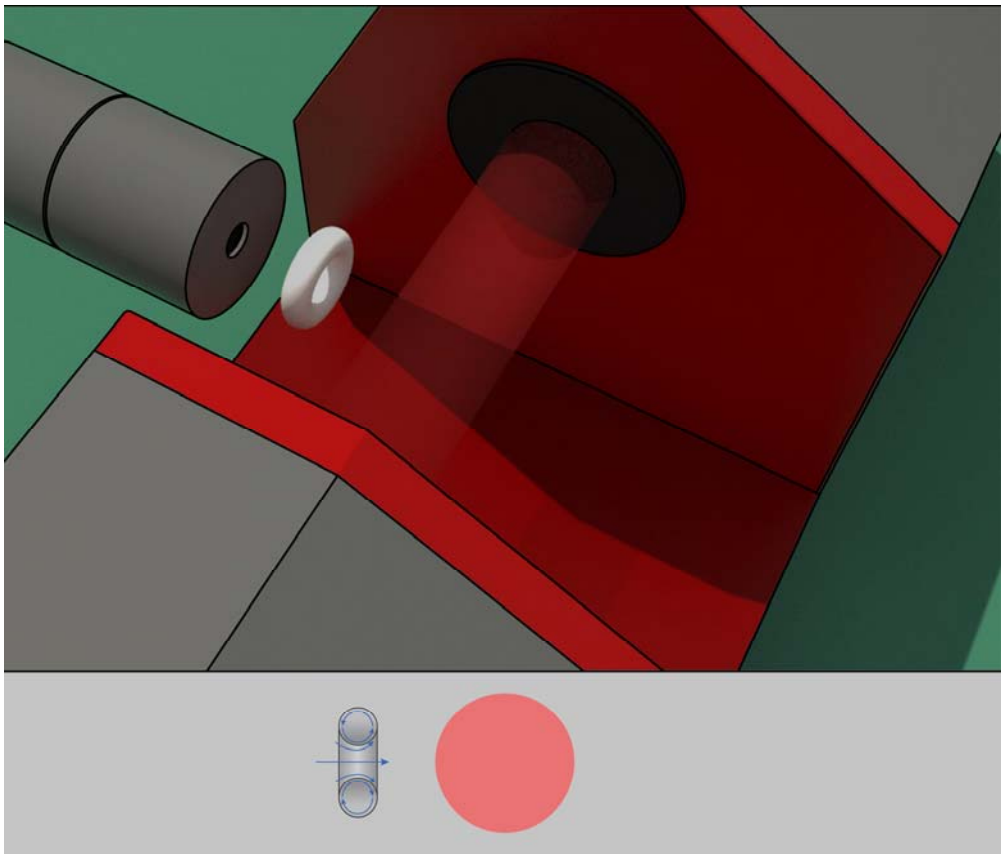

Figure 4.3. Schematic Showing the Experimental Set-up for the Droplet Size Analysis of a Toroidal Vortex by Laser Diffraction.

Plume Impaction Force

In order to assess the relative impact force generated by these toroidal vortices we custom-configured a Mettler Toledo XP26 precision microbalance. The balance was connected via serial port to a computer with data-logging software. The balance was configured into fast-host mode and balance measurements were streamed at the maximal sampling rate of 97/sec (Mettler Toledo manual). The device was placed inside the glass wind cage and an additional wind shielding apparatus/stand was built to add further protection from convective wind and to align the device. The device was actuated perpendicular to the balance plate at a distance of 3 cm in order to record direct deflections at a clinically relevant distance. Peak balance deflections were recorded and plotted vs. actuation energy to resolve the correlation relationship. See Figure 4.4 for the experimental set-up.

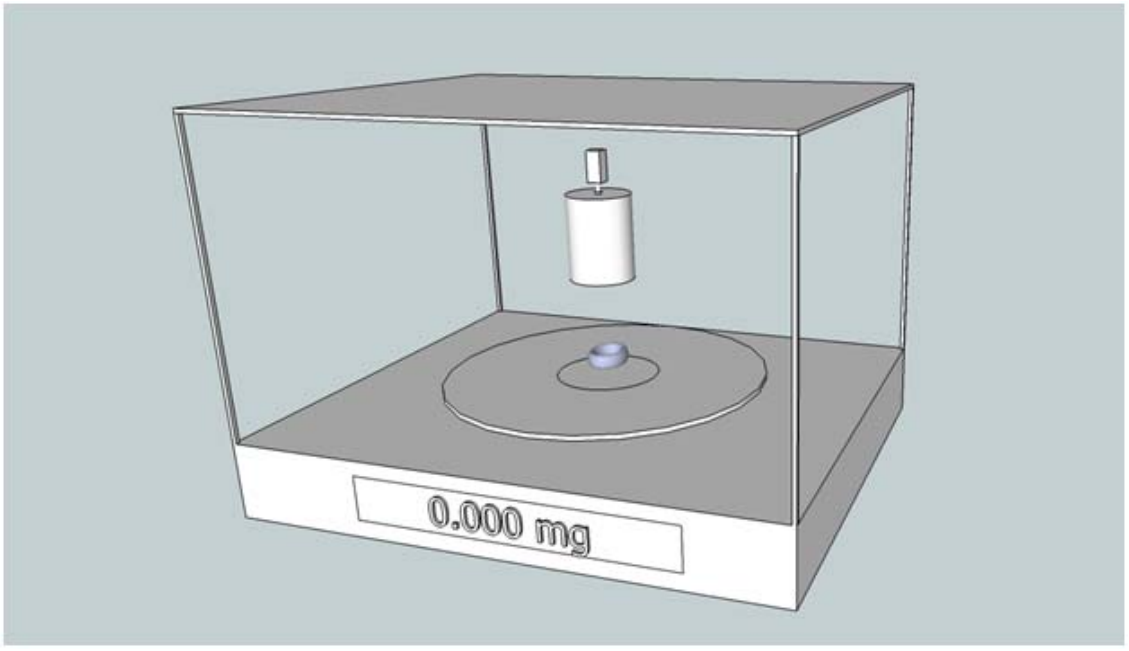

Figure 4.4. Schematic for Measuring the Relative Peak-Force Generated by Toroidal Vortices.

Results and Discussion

When designing an ophthalmic drug delivery device several key features need to be investigated in order to ensure patient safety, tolerability, reproducibility, feasibility and ultimately efficacy. The features of this device that have been identified to play a major role in the performance and feasibility of the device are related to the nature of the generated toroidal vortices and how a patient may experience them. The main features we investigate here are the components that can contribute to the patient comfort and usability. The components that contribute the comfort of administration are all related to the nature of the impact of the droplets onto the eye, the velocity and size of droplets. In addition, these parameters also influence the performance because there exists a balance between velocity and PSD for optimal deposition. Furthermore, the feasibility of the device pertains mostly in the ability to deliver the medicine before an instigated blink reflex can block or disrupt the dose (18). For this, the bulk velocity over a distance was mapped to determine if the velocity of the vortex is adequate to escape a possible blink reflex.

We found that we able to successfully characterize the major characteristics of these toroidal vortices by use of very different high precision instrumentation/techniques such as: laser diffraction, high-speed stereoscopic PIV, and microbalance real-time data sampling. Each of the methods discussed here help determine the clinical relevance and feasibility of using this device in patients suffering from various ocular diseases. While these methods are currently being investigated for applications in ophthalmology, they could also be applied to investigate other aerosol or mist devices which create very small aerosol plumes. This paper is primarily concerned with investigating the properties of these toroidal vortices and the relevant aspects the patient would directly experience, in

order to tune and optimize a final prototype device for further investigation in a clinical setting.

Droplet/Particle Size Analysis and Optical Concentration

Vortex Droplet Sizing

It is expected that the nature of the droplets generated from this device to play a crucial role in the performance, because the droplet size can affect the deposition efficiency, dose payload and even the net impact force onto the ocular surface. The manner in which these aerosols are produced from this device are very similar to that of those produced for normal pulmonary inhalation, except that these droplets are first accumulated into a loading chamber and then emitted through ambient space onto the ocular surface. When generating nebulized aerosols for pulmonary inhalation, the patient would normally directly inhale the droplets as they are being generated. And because the droplets produced from these nebulizers are very small, they are susceptible to the Kelvin effect, where the increased surface area causes an increase in the vapor pressure. This causes them to evaporate quickly when below the vapor saturation point of the atmospheric phase (19). But as they enter the environment in the lungs, where the relative humidity is near 100%, the evaporation can be reduced and even in some cases the droplets can grow due to the nonvolatile solute concentration and hygroscopicity (20, 21). In this case with a loading chamber, droplets are generated and accumulated within the chamber prior to being emitted. Therefore we suspect there will be simultaneous evaporation/condensation as well as coalescence of the droplets within the chamber and as they are emitted there could be some evaporation. To determine whether or not any of these events play a role we investigated droplet size and optical concentration as function

of both chamber fill time and distance from the orifice. As reference point we selected an intermediate or a center point for the variables in inconsideration. A chamber fill time of 3 seconds, a actuation distance of 3 cm and an actuation voltage of 9 volts was selected. The characteristics of the toroids created under these conditions are shown in Table 4-1 **)**.

Table 4-1. Droplet size data for toroids generated from a 3 second fill, 9 volt actuation at 3 cm distance.

Toroid Sample #	X_{10}	X_{50}	X_{90}	C_{opt}
Toroid 1:	1.06	2.36	4.27	11.16
Toroid 2:	0.90	2.34	4.60	10.56
Toroid 3:	0.98	2.39	4.55	14.09
average:	0.98	2.36	4.47	11.94
std:	0.08	0.03	0.18	1.89

Droplet Size and Optical Concentration as Function of Chamber Fill Time

In order to generate drug loaded toroidal vortices by this method, the aerosol chamber but first be temporarily filled. This allows for a predetermined amount of aerosol to be incorporated in the vortices upon actuation. However, the as previously mentioned, there is likely simultaneous droplet evaporation/condensation as well as coalescence. In order to assess the degree in which this may occur, we measured the optical concentration and droplet size characteristics for a fast and slow chamber fill time, corresponding to 1 second and 3 seconds. The optical concentration can serve as in indicator of aerosol concentration or number of aerosol droplets per volume. In addition, any change in droplet size would indicate if there was either evaporation or condensation occurring in the time frame in question. The results are reported in Table 4-2 . The table shows that between a 1 second fill time and a 3 second fill time there was virtually no difference in X10 and X50 fraction but showed a very small increase in the X90. However, there was a marked increase in optical concentration, from 3.01% to 11.95%. This increase is to be expected because the output rate of the nebulizer is constant and by increasing the fill time, the number of droplets/volume increases directly. These findings are important because they show that despite increasing the number of droplets in the chamber, over the course of a 3 second period, (which is much longer than a typical chamber fill) there is not any appreciable evaporation or coalescence of droplets occurring in the chamber that translates to a measurable difference once the toroidal vortex is emitted from the device.

Table 4-2. Optical Concentration and Droplet Size as a function of Chamber Fill Time at a Distance of 3 cm and 9 volt Actuation.

	1 Second Fill Time				3 Second Fill Time			
	C_{opt}	X_{10}	X_{50}	X_{90}	C_{opt}	X_{10}	X_{50}	X_{90}
	3.11	0.94	2.22	4.16	11.16	1.06	2.36	4.27
	1.91	0.83	2.07	4.09	10.56	0.90	2.34	4.60
	4.02	0.96	2.2	4.06	14.09	0.98	2.39	4.55
average:	**3.01**	**0.91**	**2.16**	**4.10**	**11.94**	**0.98**	**2.36**	**4.47**
std:	**1.06**	**0.07**	**0.08**	**0.05**	**1.89**	**0.08**	**0.03**	**0.18**

Vortex Droplet Size Over Distance

For practical purposes it not feasible to investigate the droplet sizes from within the device, and the most important feature is the size of the droplets as they approach the ocular surface. So we investigated the effect of droplet size change as the vortex propagates from the orifice to a distance of 5 cm. This distance is about the limit for practical applications in ophthalmology. As can be seen in Figure 4.5 the X_{90} for the droplets decreases as the distance from the orifice increases. More specifically, at 1 cm from the orifice the droplets have an X_{90} of ~4.25 μm and as the distance increases to 3 cm, this valued drops just slightly, but at 5 cm the droplets are noticeably smaller with an X_{90} of ~3.25 μm. To further investigate, the optical concentration as a function of time was also assessed. We found that as the distance from the device orifice increased, the optical concentration also decreased (Figure 4.6). These findings strongly suggest that there is some degree of evaporation occurring for these formulations as they propagate, but the change is relatively small over the distance of interest. Furthermore, these results have provided insight that can serve as a guide to establish proper positioning of the device relative to the eye in order to minimize variability that could be caused by evaporation.

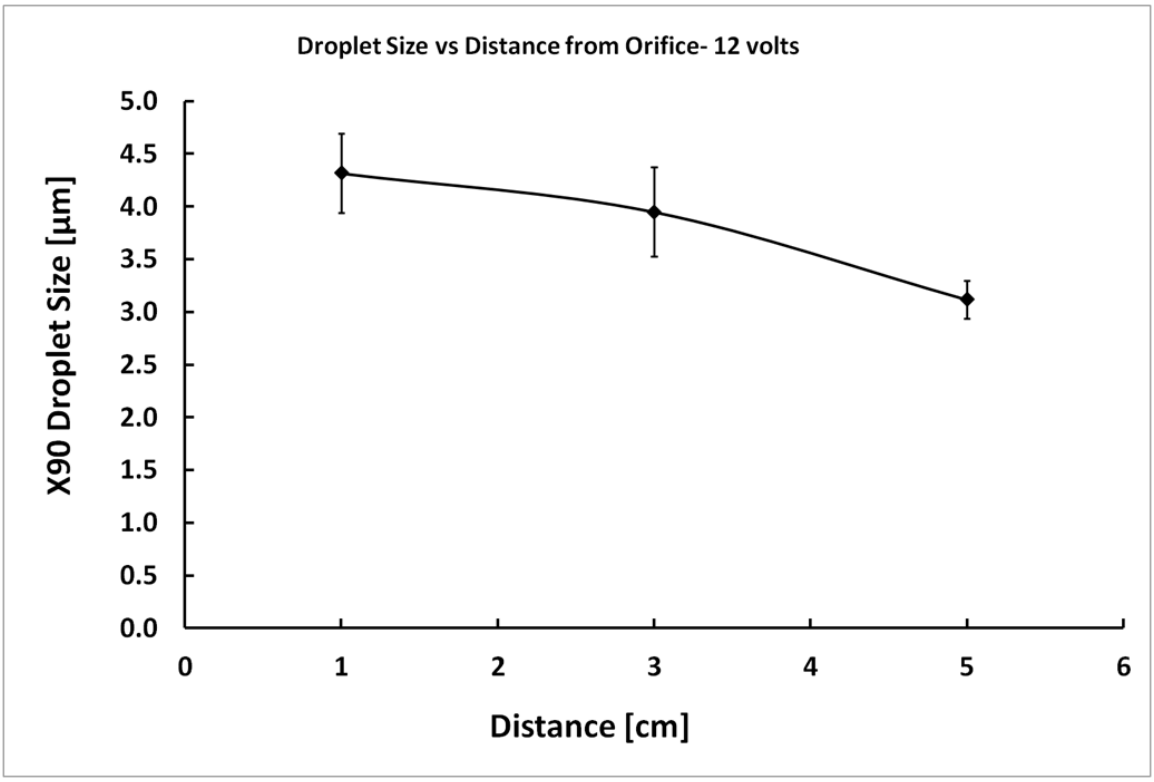

Figure 4.5. Droplet Size (X90) Decreases as the Distance from the Orifice Increases.

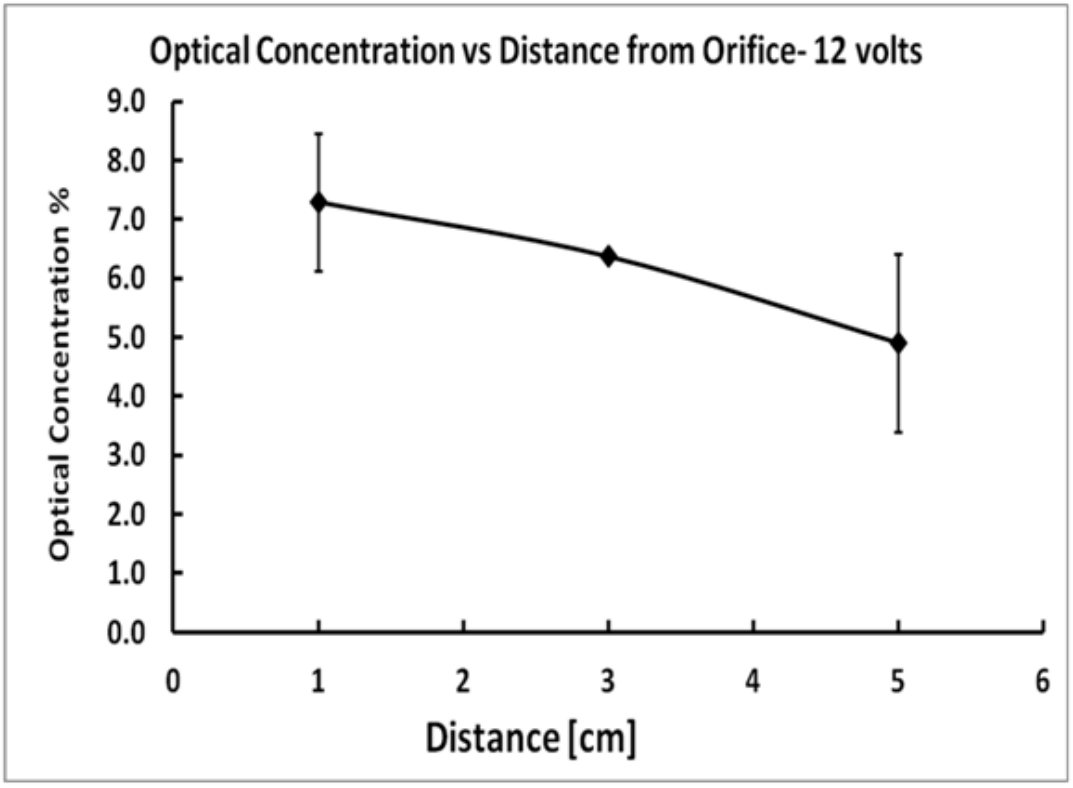

Figure 4.6. Toroidal Vortex Optical Concentration as a Function of Distance from Device.

The typical droplets produced from vibrating mesh nebulizers range on the order between 1- 5 μm and this range can shift a few micrometers to the larger or smaller end with some formulation changes. However for them most part, the droplets produced from these nebulizers are largely dependent on the nebulizer characteristics such as mesh size and energy amplitude (22). As can be seen in Table 4-1 **(**Toroid PSD-Table), when the toroidal vortex was measured directly the PSD falls roughly into this range. However, a slightly different strategy must be employed when measuring the PSD of droplets in a dynamically changing plume, and in our case we are mostly concerned with the large droplets, which carry the majority of the payload of the medicine. For reference a single 10 μm droplet can carry one thousand times more mass than a 1 μm droplet. Furthermore, it is known that upon forming a vortex some droplets can be ejected from the core filament dependent upon their size, density and the viscosity of the gaseous media (23). This could potentially cause a size segregation of the droplets spatially within the vortex and as the toroid propagates. Thus, it is important to note where the payload of the dose is located in relation to the vortex geometry because this could influence the amount of drug available for deposition onto the surface.

In order to assess this we utilized laser diffraction with ultra high speed time slicing (0.5 ms time base). This allows for the determination of the PSD of the droplets spatially within the toroidal vortex including: A) the leading edge of the vortex, B) the center point when the toroid is completely within the beam, and C.) at the trailing edge of the vortex. These special cases are illustrated in following figures respectively: **A**: Figure 4.7, **B**: Figure 4.8, **C**: Figure 4.9). Furthermore, the optical concentration was also measured simultaneously in order to assess the onset of the vortex entering the laser. A time resolved plot of optical concentration (Copt) and the X_{90} droplet size is plotted in Figure 4.10, (Labels **A** ,**B** & **C** correspond to regions of the toroid previously

mentioned.). Because the vortices are highly symmetrical, the leading edge of the vortex is representative of the outer edge or shell of the toroid. In the laser diffraction data plot in Figure 4.10, it can be seen that as the toroid moves through the beam path, there is a decrease in droplet size and as the toroid leaves the beam, the droplet size increases again. More specifically, in this example the leading edge of the toroid, Labeled A, has an X90 of 5.75 μm, while in the center it drops down to 5.15 μm, and then as it exits the size increases to 6.58 μm. This differential in size could be due to the aforementioned effect where droplets can be segregated in vortical flows based off their size and the relative velocity of gas in which they are entrained. Furthermore, it appears that the larger droplets are ejected from the core due to inertia, and occupy the out shell of the vortex, while the smaller droplets either remain in the core or drift inward due to the drop in relative pressure caused by the vortex. However, it should be mention that it is also possible that due to the increased number of droplets that are present when at the peak optical concentration, multiple light scattering events could be contributing to this observed change. Multiple light scattering cannot be ruled out completely but it is unlikely as our measurements took place below the recommended limit for Optical concentration of 15% outlined in USP<429>. Nonetheless, it can be seen directly that the PSD of the droplets are not changed to the scale that would affect their deposition and thus their performance for ophthalmic applications.

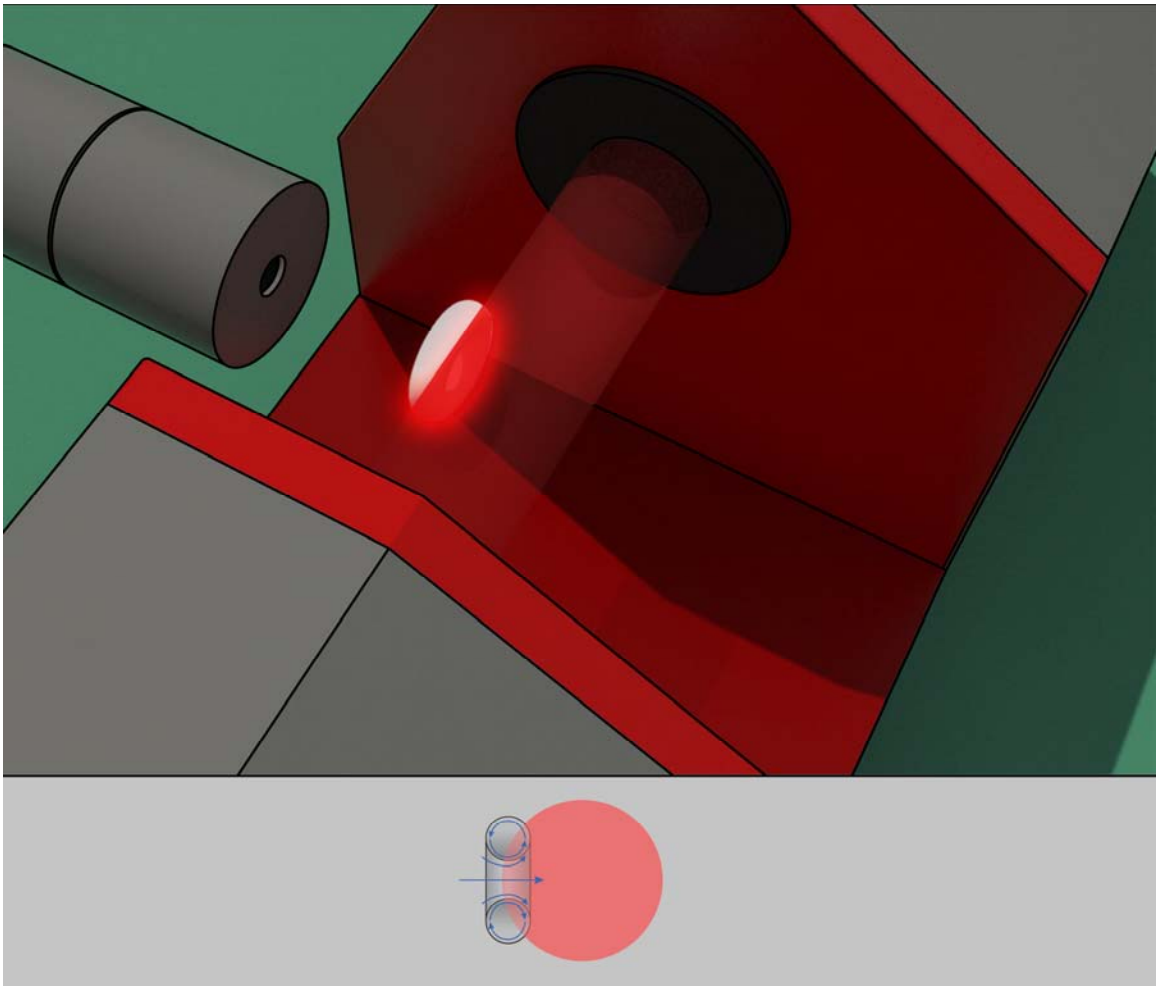

Figure 4.7. Front Edge of a Toroidal Vortex Entering the Laser Diffraction Beam

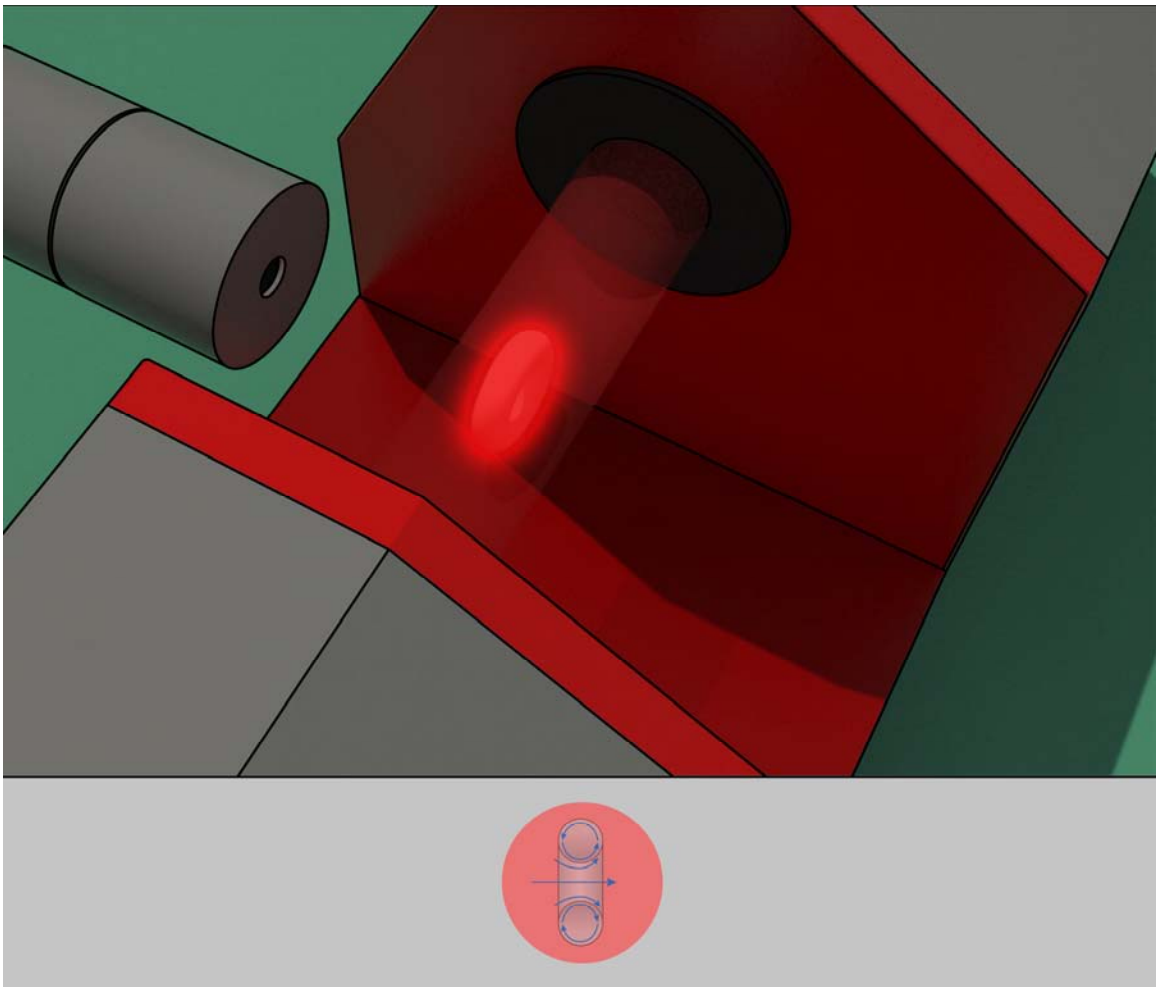

Figure 4.8. Toroidal Vortex is Entirely Inside Laser Diffraction Beam

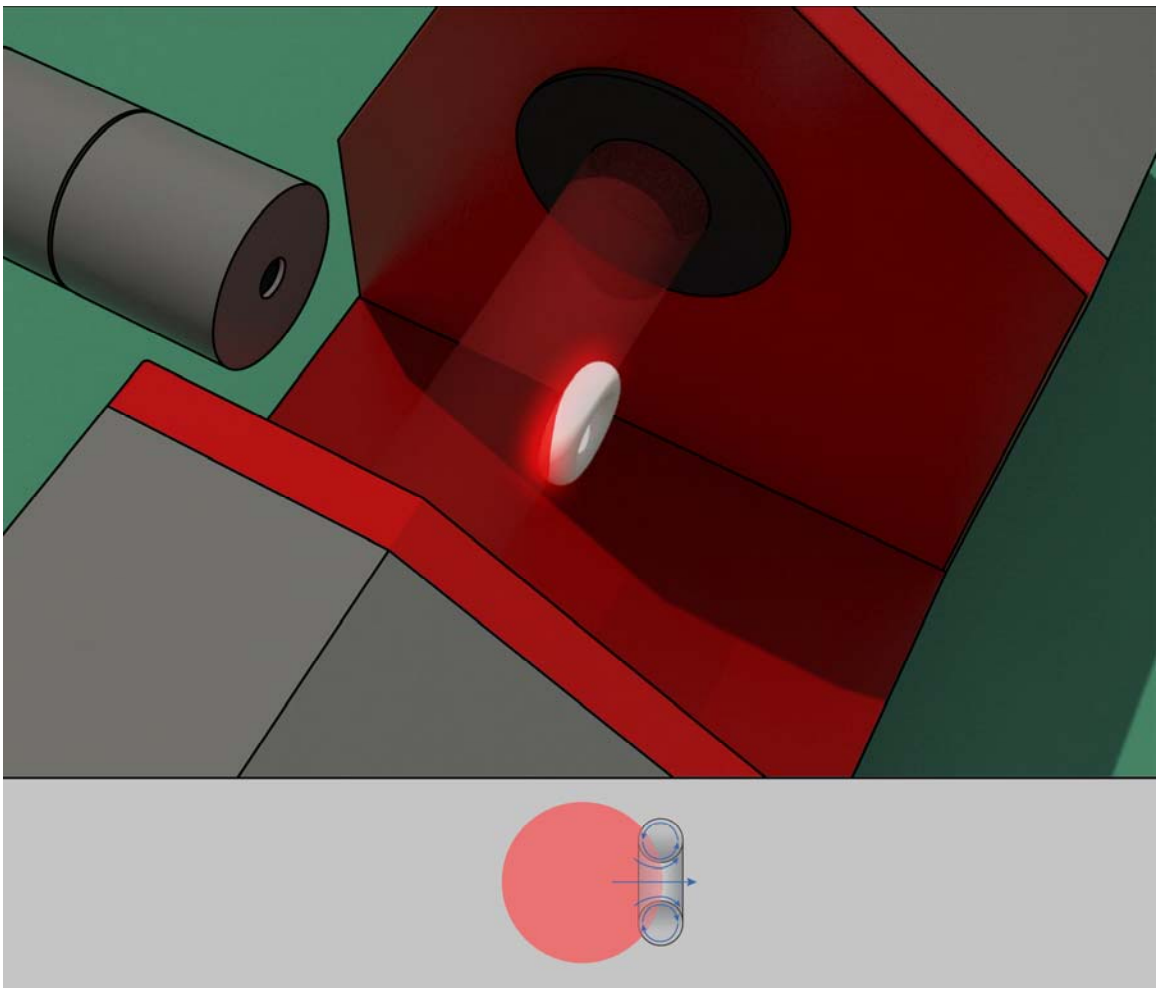

Figure 4.9. Back Edge of a Toroidal Vortex Illuminated by the Laser Diffraction Beam as it Exist the Beam Path

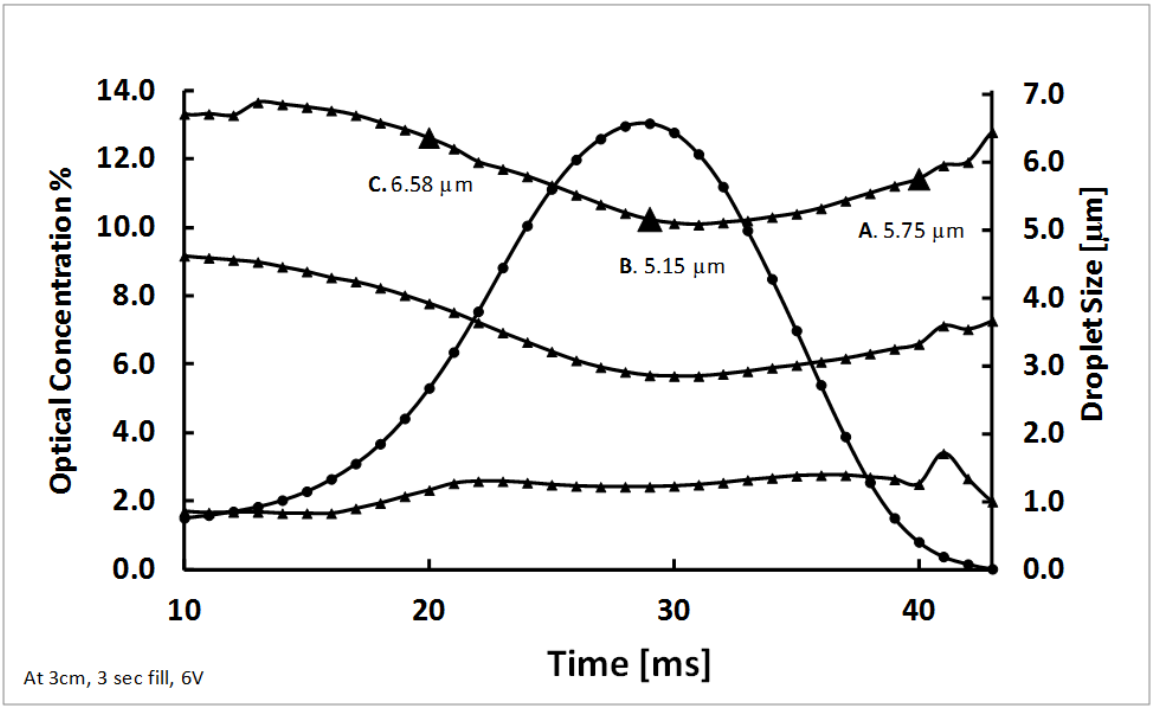

Figure 4.10. Optical Concentration plotted (solid circles), and Droplet size X_{90} (µm) (solid triangles) plotted as a function of Time. Label A.) indicates the front edge of the Toroidal Vortex. B.) When the Toroid is entirely in the laser beam path. and C.) The back/outside edge of the Toroid

Another feature that is noticeable when looking at the optical concentration-time curve from Figure 4.10, it can be seen that the signal does not return to the baseline. This is due to the lingering tail that exists during the early stage of vortex formation. These are droplets that are too large or too far away from the entrainable fluid flow to be captured into the vortex, and thus forms a streak as the vortex propagates. This streaking is also visible in the high speed video frames (Figure 4.11). Finally, from the data point at Label C) it can also be seen that the size of the droplets in the tail are larger than core, and more comparable to that of the front edge of the vortex, further supporting the notion that the radially centered portion of the vortex does in fact contain the smaller droplets (23).

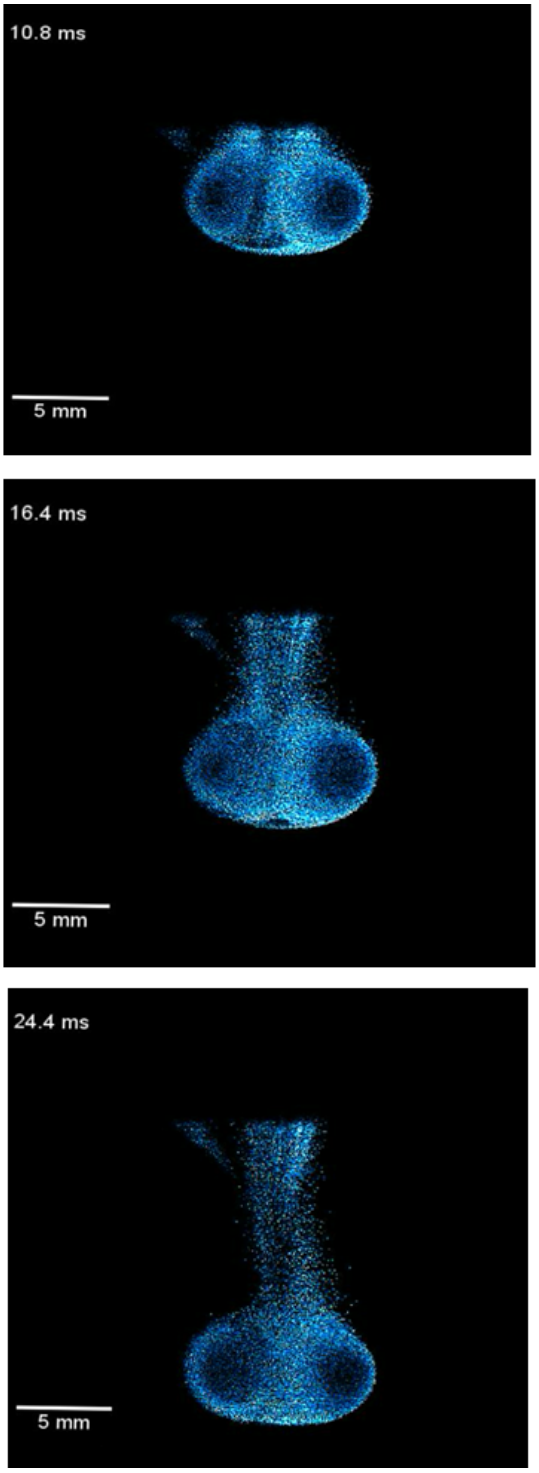

Figure 4.11. Cross-sectional View of a Toroidal Vortex Propagating at 5,000 frames per second

Toroidal Vortex Propagation Dynamics

Another critical aspect of the device is the ability to deliver the aerosol at an effective, safe and comfortable speed. A high level balance between droplet velocity and impaction force is required here, because the aerosol needs to be moving fast enough to impart adequate inertial characteristics onto the droplets so they can be deposited effectively on the eye surface without being intercepted by a possible blink reflex, while still being gentle enough to not cause discomfort.

The propagation of the toroidal vortex can be broken into two main components: (1) the bulk velocity of the vortex ring as whole (translational) and (2) the net or max droplet velocity within the vortex (rotational velocity plus translational). The bulk velocity of the plume was measured by tracking the front edge of the toroid; this provides information as to how of how long it will take for the vortex to reach the eye at a set distance. In addition, because the vortex velocity was tracked at a distance we can determine the optimal spacing from the nozzle and account for any deceleration if the eye-piece spacer needs to be adjusted.

Previous studies had shown that the velocity of an emitted vortex was proportional to the actuation force, however that velocity data was produced using a calibrated pendulum which is not feasible for patient use (16). An electronic actuator was built to impart the forces required to generate the toroidal vortices, and the control was modulated with a variable voltage supply. The device was then actuated at a low, medium and max voltage to obtain a velocity profile for each actuation energy as well as for each device over the distance relevant for delivery to the eye (~3 cm). The bulk velocity was from EZ device is displayed in Figure 4.12, and the OMR device bulk

velocity is shown in Figure 4.13. These figures, show that bulk velocities are quite reproducible and only slightly decrease over the distances measured. Furthermore, it can be clearly seen that there is a noticeable difference in the bulk velocity of toroids for each level of actuation energy, and also between devices. The EZ Device has a nearly double bulk velocity at each actuation energy than that of the OMR Device. We attribute this difference in velocity to the nature of the venting on the aerosol generator. Because the chamber is relatively sealed when force is applied to the actuating membrane, the pressure inside the chamber is momentarily built up. In the case of the OMR device, designed venting from within the device allows for this pressure build up to dissipate more readily, therefore reducing the amount of energy that is directed toward ejecting the toroidal vortex. Nonetheless, despite the geometric differences and internal venting dissimilarities in the aerosol generators, each of the devices produced very reproducible velocity profiles. Furthermore, for each of the devices, the velocities only dropped by approximately 10% from the nozzle to a distance of about 25 mm, where they would likely impact eye. This will be useful information for determining the optimal distance to set the orifice relative the eye for administration.

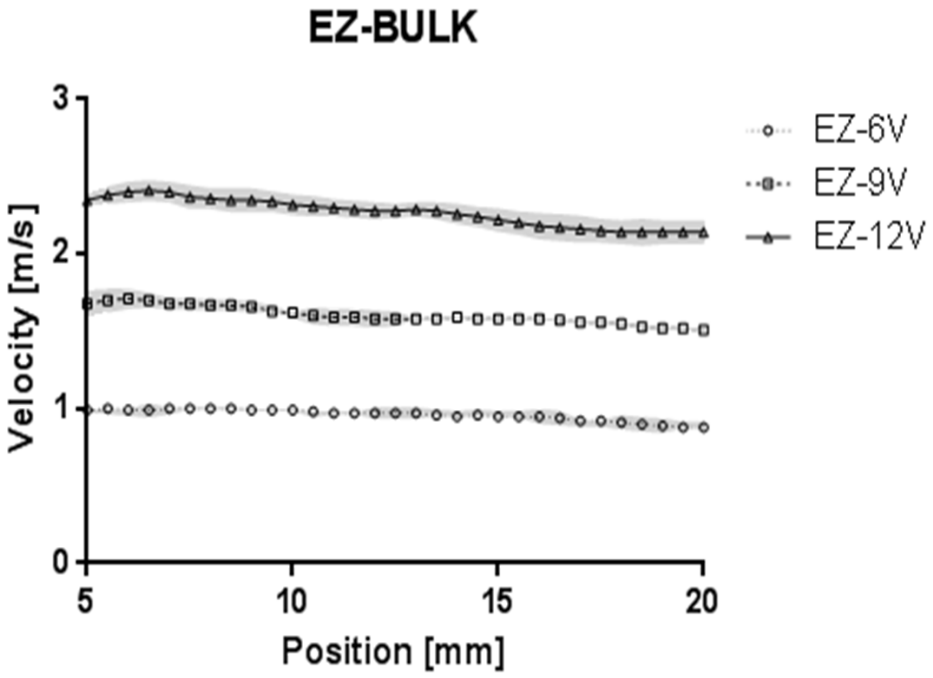

Figure 4.12. Bulk Velocity of Toroids Generated from the EZ-Atomizer Equipped Device, as a function of Distance from Device Orifice.

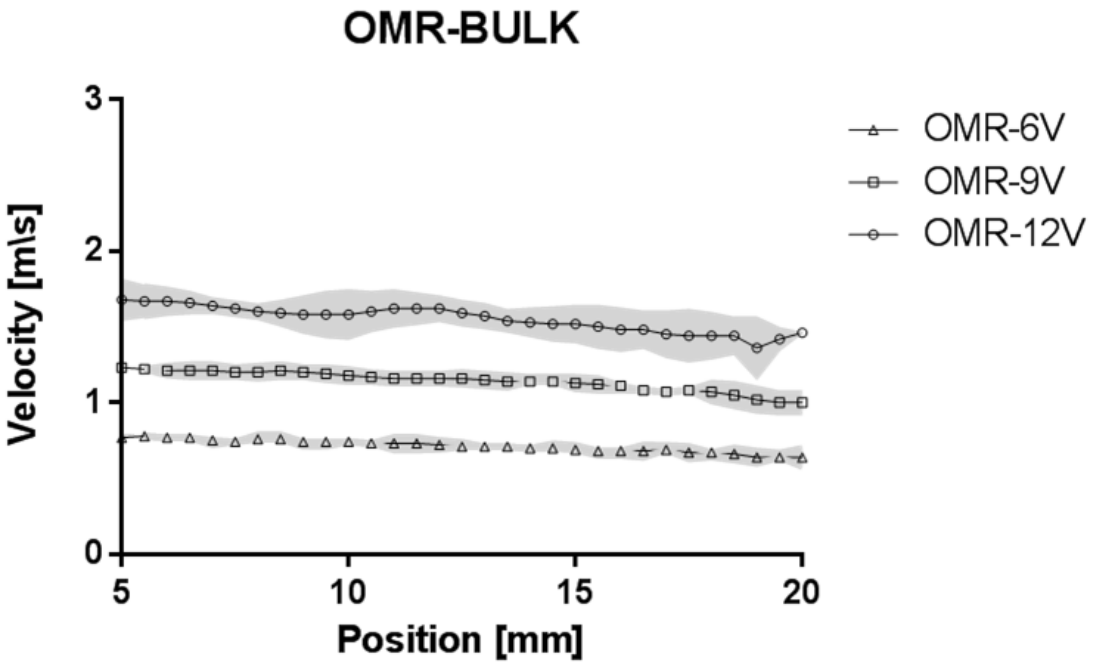

Figure 4.13. Bulk Velocity of Toroids Generated from the Omron Equipped Device, as a function of Distance from Device Orifice.

Overall, we found that in all of the cases, even the slowest moving toroid from the slowest device (OMR- at 6 volts), and the amount of time it would take to deposit, is on the order of 30 ms from actuation to contact. This is clinically relevant because the amount of time it takes for the human blink reflex is nearly 300 ms. This means that even if the actuation of the device causes a reflex blink, the toroid would have already exited the device and impacted the eye before the eye-lid could close and block the dose. However, these findings do not rule out the possibility of the patient having an anticipatory blink or "normal blink" which starts from before the device is actuated.

Beyond testing the bulk velocity of the toroids, because they are rapidly circulating it is also important to determine the maximal velocity of the droplets within the toroidal vortex. This takes into account the bulk velocity plus the rotational velocity. This velocity has major implications in terms of assessing inertial deposition and net impact force onto the eye. As can be visualized in the 2D-Velocity Contour plots in Figure 4.14, the internal core of the vortex is where the maximal velocity is located. This is because as the vortex propagates the outer walls are rotating around a circular central axis and as it rotates around towards the center of the ring, this rotational velocity is added to the net velocity of the droplets (indicated by the blue region of the 2D velocity contour plot). The opposite is the case on the outside edges of the toroid, where the droplets are actually moving in the opposite direction of toroid propagation (indicated by the red region on the 2D velocity contour plot). This contrasting velocity field has an interesting effect on the performance, because at low bulk (translational) speeds, the rotational velocity is high enough to cause a net relative velocity in the opposite direction on the outside walls. The outside edges of the toroid in (FIG>Contour plot EZ-6V) indicate a net negative velocity of the droplets. This is important because these droplets will not be available for deposition onto the surface because they will not have forward

inertia and they can be effectively subtracted from the available surface area of impaction. However, this net negative effect on the outer edges is eliminated at higher toroid velocities, thus increasing the effective impact surface area. In this case, the total forward moving velocity overcomes the rotational velocity and thus the entire toroidal surface area then becomes available for deposition, which can help to explain the observed higher dose deposition which occurred at higher speeds that was reported previously (16).

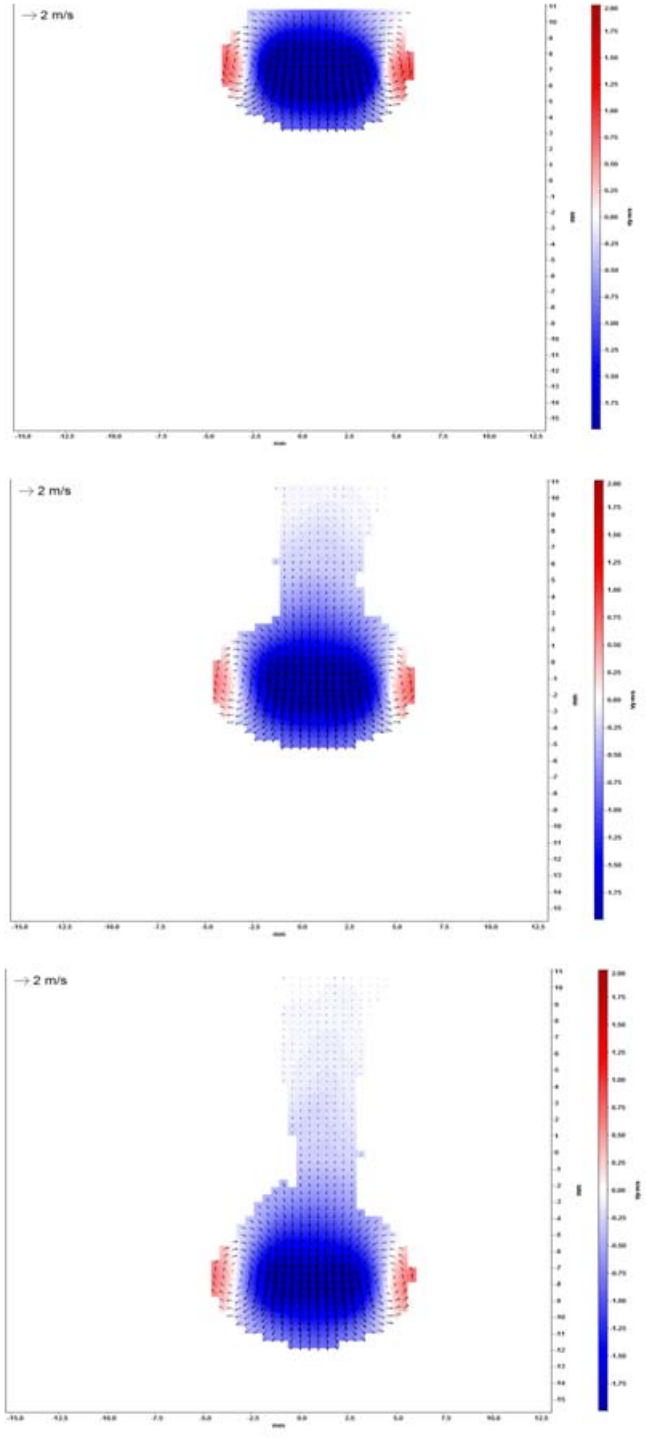

Figure 4.14. Stereoscopic Particle Image Velocimetry- 2D Contour Plots for Droplet Velocity Vectors.

In summary each of the devices tested showed a high level of precision and reproducibly when generating aerosol plumes over a range of velocities. Moreover, The EZ device (Figure 4.15), and the OMR device (Figure 4.16) exhibited nearly linear increase in bulk toroid and maximum droplet velocities across all of the actuation energies. This level of consistency and predictability will offer a higher degree of control when applying aerosol the surface of the eye.

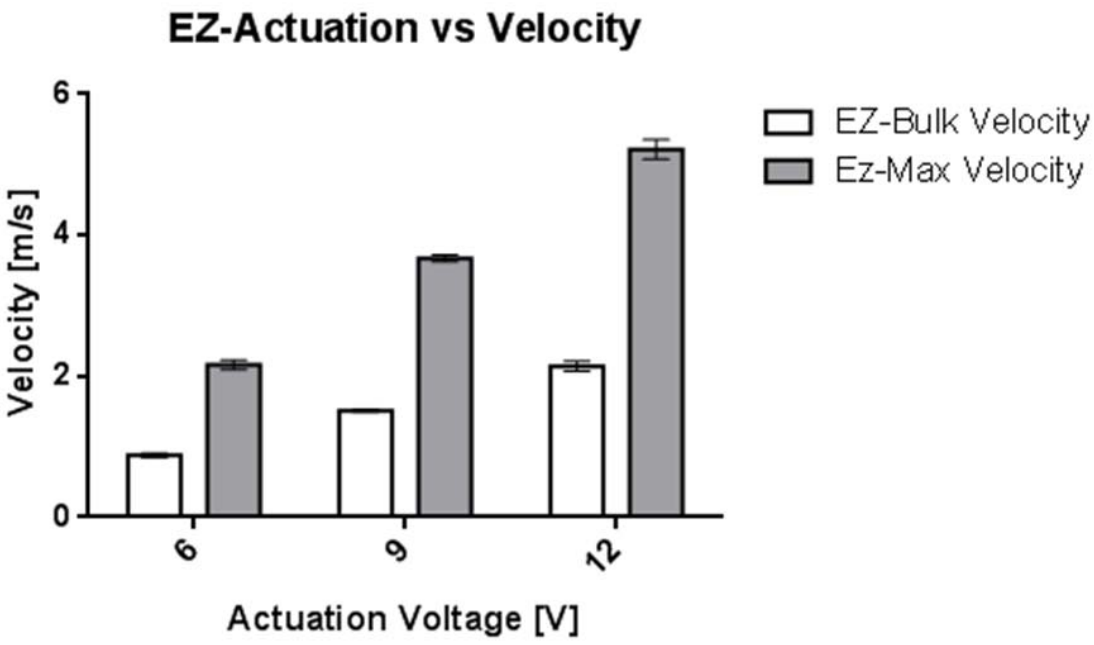

Figure 4.15. Comparison between Toroid Velocity Maximal Drop Velocity at Different Actuation Energies for EZ-Atomizer Device

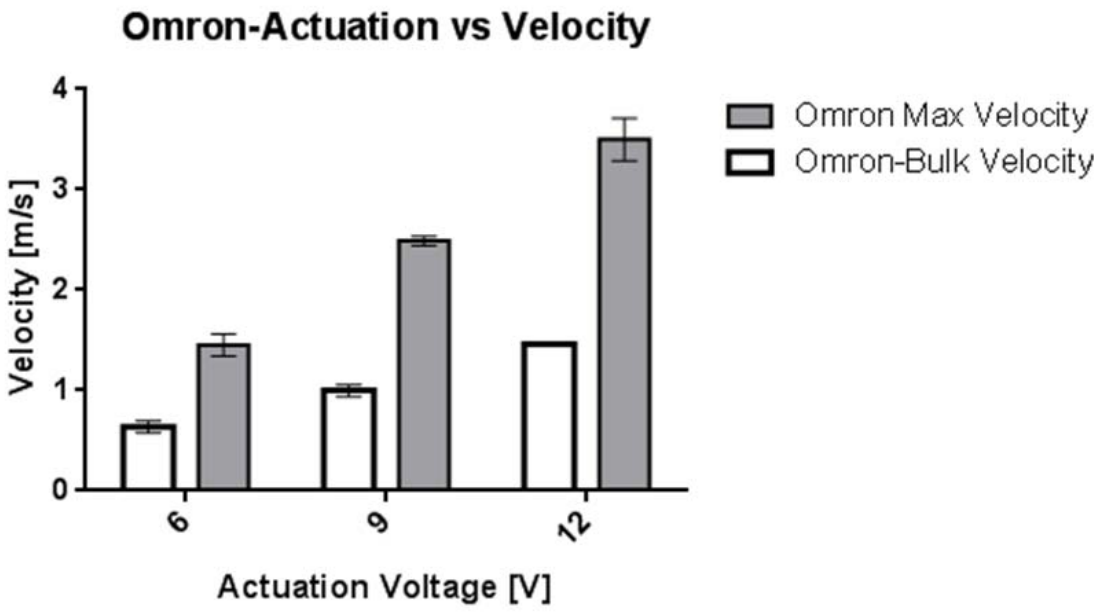

Figure 4.16 Comparison between Toroid Velocity Maximal Drop Velocity at Different Actuation Energies for OMR Device

Special Considerations regarding Uncertainty and assumptions:

During the course of making PIV measurements and collecting data, uncertainty can arise in two main ways with PIV measurements. The first uncertainty pertains to the velocity magnitudes, and it is estimated that based on the correlation statistics approach (Wieneke, 2014) that for all cases it is found to be less than 0.04 m/s and 0.1 m/s for the in-plane and out-of-plane motion velocity components (24). The second aspect of uncertainty relates to the position of the laser sheet relative to the toroidal vortex. Because the goal of the experiment is to measure the velocity in a centerline cut plane in which the vortex travels, it is imperative that the orifice is properly aligned. However, a misalignment may still exist either from the onset or via drift as the vortex propagates. In order to correctly interpret the measured velocities the stereoscopic PIV was employed. The alignment of the cameras is able to detect and obtain data from the out-of-plane velocity field.

Differences in Aerosol Generating Devices

In these experiments we used two different aerosol generating devices and attached them to the vortex drug delivery device. Both of the aerosol generators operate via the same aerosol production mechanism (i.e vibrating mesh technology), but they have different specifications. Because they typically produce aerosols with similar size characteristics, any small differences in droplet size between the devices would not likely have an effect on the velocity data.

Relative Impaction Force

During the toroidal vortex velocity and PSD testing we concluded the EZ-Breathe-AG equipped device provided the widest range of velocities as well as an adequate PSD for evaluation in the relative impact force screening. This method was developed in house to provide only in a relative sense the forces these toroidal vortices may impart onto a surface. Delving into the complexities of the impaction onto an actual ocular surface is beyond the scope of this manuscript. However, this analysis can provide guidance into evaluating the nature of the impaction and help to reduce concerns one may have about the comfort of the administration.

When deciding which device and loading scheme to evaluate for this method we had to consider the very small magnitude of the forces we were attempting to measure. Initial testing of the device without loading aerosol was found to be below the limit of detection of the microbalance. Previously Guo et al. investigated impaction forces generated from nasal sprays and pressurized metered dose inhalers using a TA Instruments texture analyzer which was turned onto its side and the doses were impacted onto the probe detector (25), however, the forces in consideration in this study were found to be much lower in magnitude than this instrument would allow. Furthermore, Muller et al. investigated the impaction force generated by ophthalmic sprays using a piezoelectric transducer at several distances. In addition, Muller et al. has also developed techniques for measuring impaction force but still none of these methods are sensitive enough to detect on the micro-Newton scale(<1 mg-F) (25-27).

The principles of force measurement between our experimental set-up and others is nearly identical however the piezo-electric transducer within the analytical microbalance is much more sensitive and allowed to data streamed so that a peak force

could be obtained. Upon actuating the device at a distance of 3 cm at low voltage only a small deflection in baseline force was measured, on the order of about 0.3mg. This was determined to be about as low as our balance would allow reproducibly. As the actuation voltage was increased the impact force was only slightly altered, until the maximal actuation force of 12 V relayed a deflection of 0.5 mg, which correlates to about 4.5 micro-newtons. The microbalance was capable of reproducibly measuring these force curves, and a typical force-time curve is reported in Figure 4.17. Figure 4.18 shows the relationship between actuation force and impact force. It was demonstrated that even at the highest actuation energy, there was only a very small increase in relative impact force.

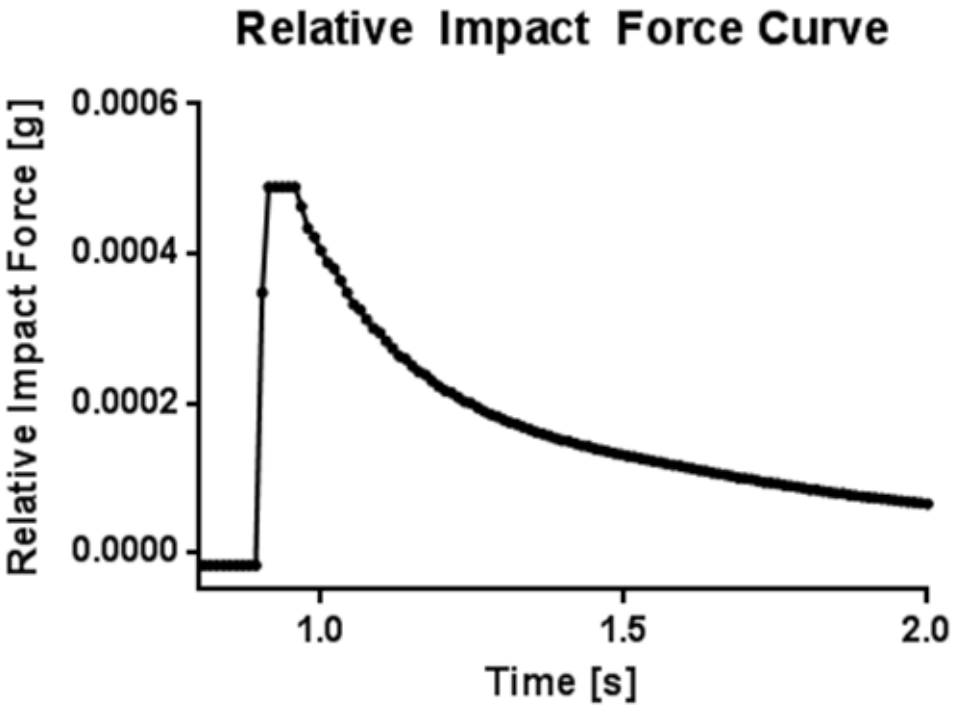

Figure 4.17. Typical Peak Relative-Force curve.

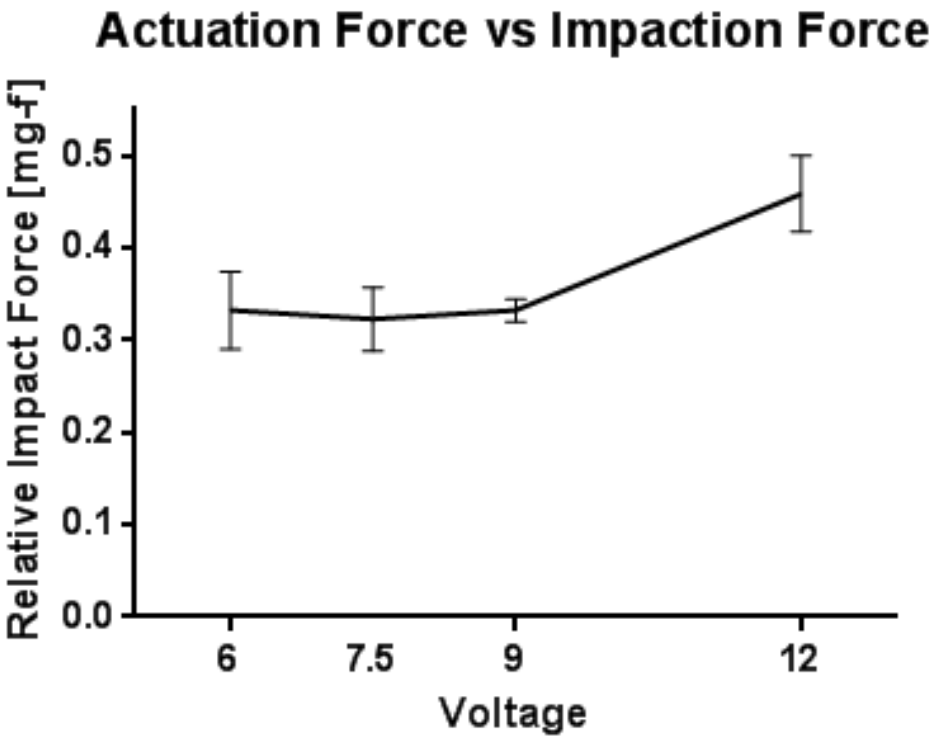

Figure 4.18. Actuation force vs Relative Impact Force

Previously Muller et al. showed that when comparing an eye drop to an eye spray, there was an inverse relationship between the distance of application and the force for the spray and a direct relationship between impact force for drops and distance. In essence the drops gain momentum and impact harder the further they are away, while the spray loses velocity the further away it administered. While this decrease in impact force from distance is preferable, a drawback with spray type devices is that they emit aerosol in a cone-type geometry, meaning that the further away from the device the larger the diameter of the cone. Muller et al. found that at just a distance of 1 cm the diameter of the cone was ~25 mm, not only is this larger than the diameter of the exposed portion of the eye, this distance corresponds to impact force of about 10 mN. This impact force corresponds to about a 1 gram-force, nearly 2,000 times the forces generated from the toroids found in our studies and triple the force created by an eye drop at the same distance. At an equivocal distance of 3 cm, the spray force from a spray is reduced ~5 milli-N, but at the cost of increasing the diameter of the spray cone to about 35 mm, where a large portion of the spray is likely to miss the ocular surface.

Uncertainty in these measurements exists in the orientation of the device in that if the toroidal vortex does not directly impact the plate the results will be slightly different. In addition, if the vortex hits off center the pan this will cause a competing torque force on the lever arm. These results will be untrue to the method. Nonetheless, for the purpose of demonstrating the relative scale (semi-quantitative analysis) of the impaction forces as they pertain to the clinical relevance of this device this method was demonstrated to be satisfactory.

CONCLUSIONS:

In summary, we found that despite the large differences in velocity both bulk and maximal droplet velocity the resulting impact forces generated from these toroidal vortices changes very little and this likely due the small mass contained in each vortex. Furthermore, it was determined this impact force is far below the threshold for what a patient could tolerate relative to other forces currently used clinically in patients. In addition we also measured the size of the droplets to be on the order of which would be suitable and comfortable for ocular drug delivery and that the delivery of the bolus would likely out-pace reflex blinking if it were to be triggered by the device. These preliminary studies have offered support for advancing the investigation into a pre-clinical setting and much more testing would need to be conducted to show the performance, safety and efficacy of the delivery device in animal and human subjects.

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SECTION 3: FORMULATION STRATEGIES

Chapter 5 : Solubilizing Agents for Improving Drug Loading into Aerosolized Ophthalmic Toroidal Vortices

ABSTRACT

Recently there has been an increased interest in the use of liquid aerosols to deliver medicine to the surface of the eye particularly due to their small volume capabilities. We have developed and previously described a precision aerosol device for ocular delivery. In this article we investigate the use of two different excipients, hydroxypropyl-β-cyclodextrin and glycerin, for use as solubilizing agents and for modifying the rheology for use in an ocular aerosol formulation. We found that while both excipients contributed a comparable amount to the dynamic viscosity of the formulations by weight, they had dramatically different ability to solubilize the active ingredient, diclofenac sodium. Furthermore, HPBCD negatively affected the aerosol output rate but not to an extent that overcame the higher solubilization capacity benefits. Finally we found that neither of the vehicles significantly altered the droplet size distribution across a wide range of concentrations.

INTRODUCTION

Recently there has been an increase in the prevalence in ocular related disorders and this only expected to increase with an aging population. As of 2013, there was an estimated 64.3 million patients affected by glaucoma, which is expected to increase to 76

million by 2020, and even to over 110 million by 2040 (1). This new era of medicine requires new outside the box thinking in order to solve some of these long-standing problems with ophthalmic treatments. While there have been major developments in creating new drug entities to treat various ophthalmic conditions there still remains major barriers to getting the medication to the target tissues. Using conventional dosage forms such as eye-drops, it is estimated that less than 5% of administered medication makes it to the target tissues, and in some cases less than 1% (2-4). Other problems associated with eye drop include poor patient compliance, ease of use and unfavorable systemic side effects (5, 6).

The biological challenges associated with topical ocular drug delivery mostly depend on either penetrating the physical barriers such as the cornea, sclera and uvea. But also in overcoming the physiological aspects of the ocular system such as tear production/rinsing of the surface with enzymes that degrade drug molecules before permeating the ocular membranes. Without modifying the chemical active (as in the case of prodrugs) scientists are limited to the addition of excipients and altering the dosage forms. The main strategy for increasing the bioavailability is to increase the permeation of the drug or the residence. To increase the permeation of the drug scientists have used several techniques such as, permeation enhancers which can alter the membrane barrier to promote absorption. Also scientists can increase the concentration gradient, within the tolerable limits of the eye to promote diffusion. Another strategy to increase to bioavailability is the targeted use of transporters to either increase the drug influx or reduce/block the efflux across key barriers (7).

Aside from altering the dosage form to improve permeability, scientists have also made attempts to increase the residence time at the surface of the eye in order to increase the extent of absorption. Some of these strategies include increasing the viscosity of the

instilled liquid, as in the case of in-situ forming gels or ointments. Blurring vision and difficulties in application may limit widespread use of this approach. The use of drug loaded ocular inserts, contact lenses, punctal plugs etc. can also greatly improve the residence time at the interface of the eye. However, issues with administration and ejection also pose a challenge with these systems in their current design.

As early as the 1970's scientist learned that instilling eye-drops in small volumes could reduce clearance from the surface of the eye and increase the bioavailability and further reduce the systemic effects (8, 9). It was only a few years later when scientist began incorporating these small drops into sprays or mists to deliver the liquids to the eye(10, 11) . While they were demonstrated to be equivalent in many cases despite a smaller delivered dose, this technique did not catch on due to variability in the plume spray and in ability to direct it onto the eye and not the surrounding tissues. More recently, companies such as Mystic Pharmaceuticals and Eyenovia have developed devices to administer sprays onto the eye in a more controlled manner.

As mentioned previously, the formulation of aerosols for topical administration to the eye has a separate set of limitations as to that of typical eye-drops or solutions for inhalation. When depositing aerosols (micronized droplets) onto the eye, a very small volume of liquid is actually instilled. Therefore this requires a much higher concentration of active ingredient at least 10X what is typically used in eye drops. This high concentration of solubilizing agent can work synergistically by increasing the viscosity of the liquid to promote retention on the ocular surface. However, this increase in concentration can alter the physicochemical properties of the liquid such that the aerosolized medication is compromised. Therefore we must determine the optimal concentration of cyclodextrin or glycerin that will allow for maximal dosing without

reducing the aerosol output from the aerosol generator and shifting the droplet size outside of a usable range.

Early research by several groups showed that the drug permeability across the corneal membrane could be directly increased by loading the drug into cyclodextrin complexes and by possibly altering the structure of the corneal epithelia (12-14). Cyclodextrins are cyclical oligosaccharides that have different hydrophilic functional groups exposed on the outside surface and lipophilic groups in the core on the inside, where a lipophilic drug can be solubilized. The cyclodextrin complexes work in two main ways. Firstly, they increase the solubility of the drug in the aqueous phase thereby increasing the availability of the drug at the membrane for diffusion. And secondly, they are reported in some cases to alter some membrane constituents and thus change the permeation characteristics of the membrane(15). In the case of solubilizing lipophilic drugs, the solubilized drug or uncomplexed drug can diffuse more readily across the membrane (16). This solubilization technique is widely used in other drug delivery methods/routes as well as in investigational ophthalmic applications, and cyclodextrins are used as the main solubilizer in the Voltaren® Optha eye drop formulations already approved for use in the European Union (17).

MATERIALS AND METHODS:

Materials:

Phosphate buffered saline was purchased from Sigma-Aldrich. Hydroxypropyl β-cyclodextrin (Cavasol® W-7 HP) was graciously donated by Wacker Chemie AG. Glycerin and Sodium Diclofenac (DCN) were purchased from Fisher Scientific. De-ionized water (in house). Finally, an Omron MicroAir Vibrating mesh nebulizer, which was generously donated from Omron Healthcare Inc.(Wake Forest, IL)

Methods:

Cyclodextrin complexation and aqueous cosolvent formulations were prepared by shake-flask phase solubility methods set forth by Higuchi and Conners (18). Each formulation was set on shaker and equilibrated for 24 Hrs and filtered through a 0.2 um PTFE syring filter prior to analysis. To determine the amount of diclofenac sodium solubilized by HPBCD the samples or the co-solvent system, placebo formulations were used as blank with the corresponding formulation and diclofenac loading was quantified by UV-Vis spectroscopy using a Tecan M200 spectrophotometer, measuring absorbance at a wavelength of the 276 nm.

Output Rate:

The nebulizer output rate/aerosol generation rate was determined gravimetrically in triplicate for 5 second intervals of time for each formulation. In addition the supplementary measurement of optical concentration was done using the laser diffraction

apparatus to give an indirect measurement for droplet concentration which could indicate nebulizer output rate.

Droplet Sizing:

Particles/Droplet Size was measured using a Sympatec Helos Laser Diffraction Apparatus with the open bench set up. The LC 15mm focal length lense was used at set distance of 100mm. The particle size distribution by volume was calculated using HRLD and the key outputs from the report form are X_{10}, X_{50} and X_{90}. Finally, the optical concentration was also measured simultaneously as an indirect means for correlating droplet concentration to the nebulizer output rate.

Kinematic Viscosity:

The kinematic viscosity of each of the formulations (n=3) was measured using a Cannon-Fenske, glass capillary flow through viscometer equilibrated at RT for 30 minutes prior to measurement.

Complexation Constant: $K_{1:1}$

To determine the binding affinity between the diclofenac and the HPBCD, the solubilization capacity was plotted vs the molar amount of cyclodextrins. The formula for the complexation constant $K_{1:1,}$ is given by Equation 1, below(19). Where the slope, is the slope of the plot for solubilized diclofenac Na vs Molar Concentration of HPBCD. And $D_{0,}$ is the baseline equilibrium solubility for diclofenac Na.

Equation 1:

$$\mathrm{K1{:}\,1} = \frac{Slope}{D0(1 - Slope)}$$

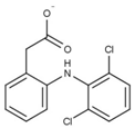
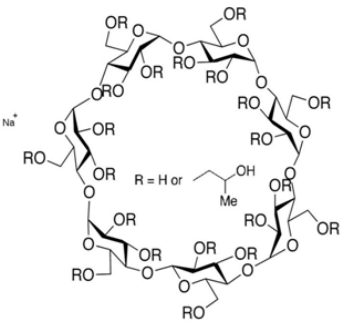
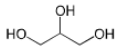
Preparation of the Solubilized Formulations:

Cyclodextrins have widely been studied for use in the eye as solubilizers, penetration enhancers, stabilizers and are approved for use in eye drops in Europe (20, 21). While they have been formulated for use in eye drops, this is the first of our knowledge where highly concentrated solutions have been nebulized into micro-droplets for ocular use. When formulating aerosols for ophthalmic drug delivery a different set of concerns need to be taken into consideration, as the nature of the delivery of the microdroplets is slightly different. The main difference is that the total volume of liquid that is being added to the tear film is dramatically reduced from about ~50 µl to less than 1 µl. This has ramifications because the there is a physical limit to how much drug can be loaded into this very small volume and in this case we are attempting to maximize the dosing so that at a later time if need be the dosing can be reduced. The limits of the drug solubility ultimate dictate how much drug can be incorporated into the droplets. On the other hand a couple of the normally limiting aspects of drops can also be avoided. A table (Table 5-1) of the relevant physicochemical properties is shown below.

In this very specific case, because of the very small instillation volume, it necessitates that the solution is much more concentrated than a typical eye drop. And because diclofenac has limited solubility in aqueous solutions, higher amounts of solubilizing agent must be incorporated in order to achieve adequate dosing.

Balancing the incorporation of a poorly water soluble drug into a formulation which is still capable of being aerosolized with the desired characteristics is a challenging task, especially at higher concentrations of solubilizer. A normal eye-drop formulation is not held to these limitations but the normal eye-drop also does not have very stellar performance either, sometimes <1% bioavailability.

Table 5-1. Physicochemical properties of Key Formulation Components

Physicochemical Properties			
	Diclofenac Na^+	Hydroxypropyl-β-Cyclodextrin	Glycerin
Chemical Structure:			
Molecular Weight	318	1400	92
Melting Point (ºC)	283-285	120-160	17.8
pKa	4.2	-	14.4
log *P o/w*	4.4 (exp)	-	-1.76
S_o (mg/mL) in water	5.2 (pH 7.4)	~2,300	Miscible

Complexation Binding Constant:

The ability of a cyclodextrin to solubilize a drug molecule depends largely on the geometry of the molecule and physicochemical characteristics of the drug. In this, Diclofenac Na, has pH dependent solubility but is not soluble enough for the high concentrations needed in aerosol delivery. It also has a high log P, which makes it a good candidate for solubilizing with cyclodextrins, because the core of the HPBCD is hydrophobic. When formulating with cyclodextrins it is important measure the binding coefficient for the complex. The binding coefficient mathematically describes how tightly the drug molecule is incorporated into the complex. While high binding coefficients mean high solubilizing capacity they also indicate that drug may be too tightly bound to the complex to be liberated for absorption. On the other hand very low binding coefficients correlate with very low solubilizing capacity and typically, high amounts of excipient are required to in order to achieve adequate concentrations. Diclofenac Na was found to have a $K_{1:1}$ of ~105 which is in the feasible range outlined by Rao et al.. See Figure 5.1., below, to observe the linear relationship between CD concentration and solubilized DCN. Others have investigated the use of cyclodextrins with diclofenac in ophthalmic applications (22, 23). However, in these experiments the required target concentrations to be delivered are much higher than those previously attempted to apply in eye drops because this delivery method deposits the drug in very small volumes of liquid. (For reference the commercial Voltaren® eye drops are only 0.1%)

Increasing the solubilizing agent improves the solubility and thus the loading into the aerosol droplets however there is a trade off on quality of the aerosol produced at some point the output rate is compromised so that in the same amount of time (chamber

fill time) less actual drug is processed into droplets therefore reducing the net payload of the device.

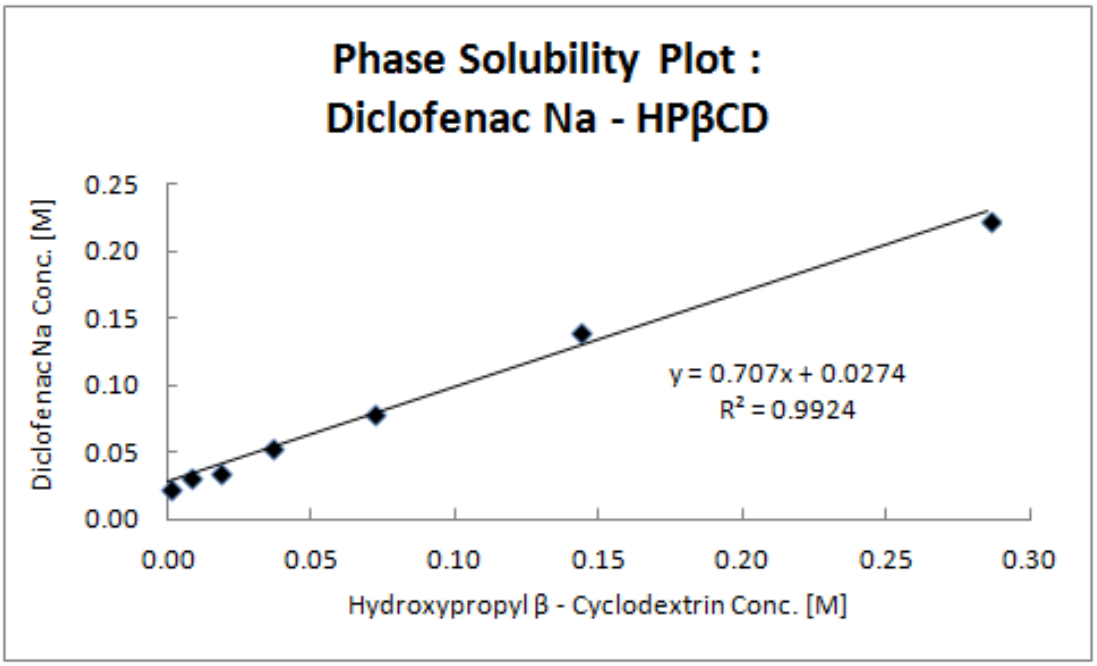


Figure 5.1. Phase Solubility Plot of Diclofenac Sodium in HPBCD

Advantages Over Eye Drops:

For one, when adding very concentrated, small volumes of liquid to the small volume of the existing tear film, theoretically, there is not a large change in volume to stimulate tear production, so the dilution of tear film over time can be minimized. In the case of an eye-drop instillation, the pre-corneal space is over-filled, and the entire tear film is virtually replaced with the eye-drop. And because the surface of the eye is very sensitive to changes in fluid volume and temperature, as well as the osmolarity and pH, the incoming formulation must be precisely balanced. Otherwise, tears will be rapidly secreted in order to re-establish equilibrium, thus washing away the dose from the ocular surface. In micro-droplet delivery we are essentially depositing the solubilized drug material and a few excipients directly into the existing tear film, thereby minimizing the role of osmotic agents and pH modifiers. Furthermore, the use of preservatives in normal eye-drop formulations has been an emerging problem for use in chronic therapies since we have learned of their corneal toxicities. When using microdroplets, the concentration of the preservative can be used the same concentration used to preserve liquids at room temp; however, the actual exposure to the patient is dramatically reduced due to the lower overall volume of liquid administered to the patient.

Kinematic Viscosity:

The major physicochemical property that affects the ability for these pharmaceutical solutions to flow is the kinematic viscosity. While the degree which the

effects of viscosity on performance are highly dependent on the aerosol generator mechanism, there exists an upper limit in which these liquids will no long flow freely and become nearly impossible to aerosol regardless of the mechanism. In vibrating mesh aerosol generators it is crucial for the fluid to be able flow through the micro-drilled holes that create the mesh. The literature suggests that the range of droplets capable of being produced is bound by the size of the holes in the mesh (24). However, interactions between the micro-orifices and liquids with different physicochemical properties can yield slightly different droplet size distributions and ultimately alter the rate at which droplets can be generated (24-26). In both cases with increasing concentration of solubilizer there was increased viscosity but HPBCD showed the highest increase at the upper loading concentration. A comparison of the kinematic viscosities of the vehicles at different concentrations is shown below in Figure 5.2. Furthermore, when incorporating DCN, this did not have an effect on the kinematic viscosity beyond that of the vehicle alone (Figure 5.3)

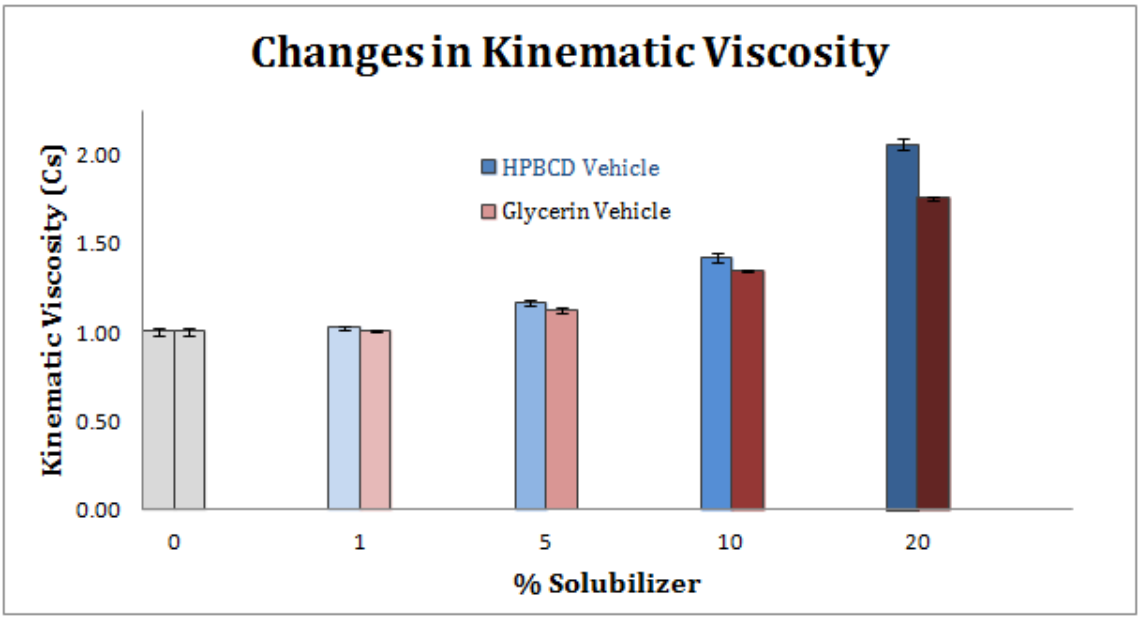


Figure 5.2. Plot Showing Kinematic Viscosity Increases as Vehicle Concentration Increases

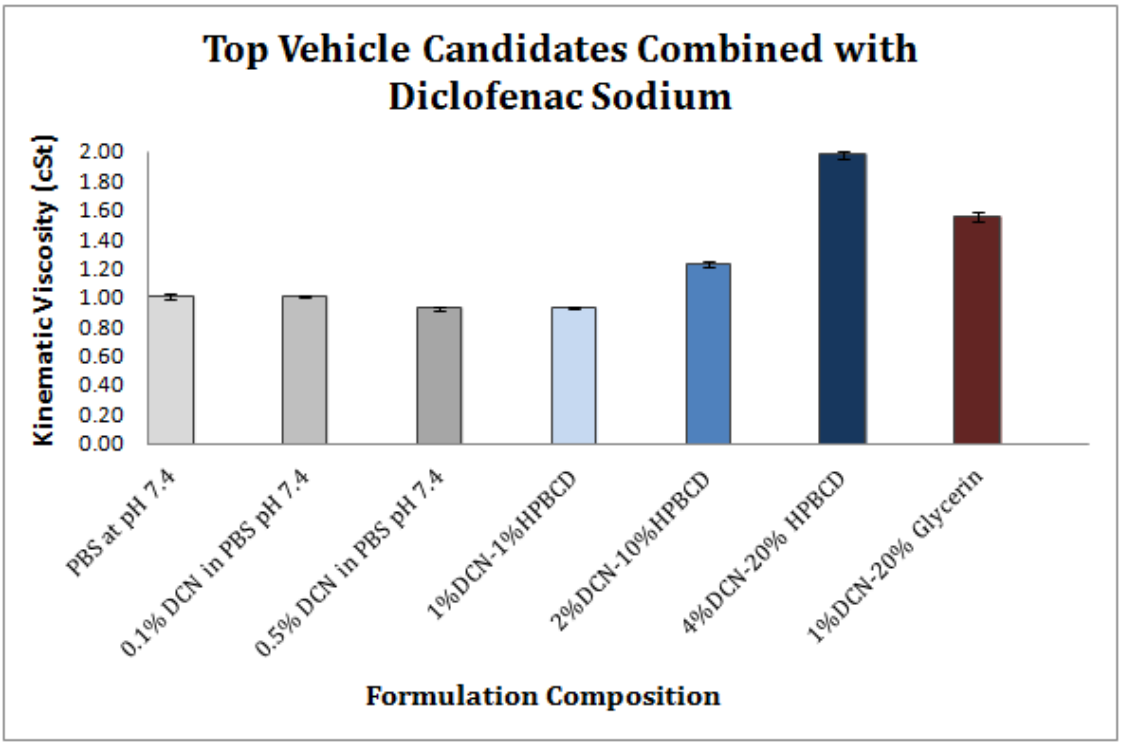


Figure 5.3. Kinematic Viscosity of Top Solubilizer Formulations

Droplet Size Distribution:

We found that despite large changes in formulation composition the droplet size distributions for each of the formulations almost had no effect. A summary of the droplet size distribution characteristics is displayed in Table 5-2. Others have reported small changes in PSD for different physical chemical properties of the solutions, however others have also reported on the robustness of the performances of the vibrating mesh technology (25). Having reproducible droplet size from this ophthalmic aerosol device is crucial as the momentum of the droplets directly related to their diameter (e.g. mass) and velocity. In addition, maintaining a consistent droplet size distribution is important because the overall payload of the dose is heavily dependent on size and number of the droplets and the more consistent and reproducible, the better to the delivered dose uniformity and ultimately the reliability of the delivery technique.

Table 5-2. Droplet/Particle Size Distribution and Optical Concentration Data for Each Solubilizer Formulation.

Characterization of the Particle Size Distribution					
Sample Name	**X(10)**	**X(50)**	**X(90)**	**VMD**	**Opt Con.(%)**
PBS at pH 7.4	1.14 µm	4.93 µm	11.73 µm	5.82 µm	44.62%
1% HPBCD	1.26 µm	4.88 µm	11.37 µm	5.74 µm	13.07%
5% HPBCD	1.00 µm	4.68 µm	9.94 µm	5.20 µm	23.79%
10% HPBCD	1.15 µm	4.83 µm	10.40 µm	5.46 µm	13.66%
20% HPBCD	1.13 µm	4.06 µm	8.69 µm	4.66 µm	10.90%
1% Glycerin	1.15 µm	5.15 µm	12.58 µm	6.17 µm	43.58%
5% Glycerin	1.19 µm	5.09 µm	12.37 µm	6.11 µm	26.28%
10% Glycerin	1.23 µm	4.83 µm	11.64 µm	5.77 µm	21.37%
20% Glycerin	1.17 µm	4.26 µm	9.90 µm	5.03 µm	23.41%
0.1% DCN in PBS	1.10 µm	4.89 µm	9.98 µm	5.36 µm	26.93%
0.5% DCN in PBS	1.07 µm	4.97 µm	10.57 µm	5.57 µm	30.59%
1% DCN -1% HPBCD	1.10 µm	5.01 µm	10.66 µm	5.62 µm	28.18%
2% DCN -10% HPBCD	1.13 µm	4.52 µm	9.53 µm	5.06 µm	12.40%
4% DCN -20% HPBCD	1.12 µm	3.76 µm	8.64 µm	4.49 µm	6.17%
1% DCN -20% Glycerin	1.18 µm	3.56 µm	7.59 µm	4.10 µm	14.83%

Nebulizer Output Rate:

One of the main ways the dosing of this device is tuned is via the chamber fill time. Increasing the mass of aerosol (i.e. # of droplets) into the chamber has very predictable and reproducible effect on drug deposition after being emitted from the device. So in this regard, the nebulizer output rate directly influences the amount of time needed to adequately fill the device aerosol chamber prior to actuation. We found that the amount of solubilizing agent used had a smaller effect on the aerosol output rate than just the neat addition of the drug to the phosphate buffered saline solution (compare Figure 5.4 (solubilizers alone) to that of Figure 5.5 (solubilizers plus DCN). The normal output rate for PBS was ~5 mg/sec, and adding the diclofenac to the formulation at a concentration of 0.1% (w/v), reduced the aerosol output down to about 1.2 mg/sec but further increasing the concentration to 0.5% only did not noticeable affect beyond the initial drop. This effect could possibly be explained by the surface active properties of diclofenac, and its interaction with the vibrating mesh (27, 28). Futhermore, compared to the vehicle solutions without diclofenac, the incremental addition of glycerin increased the kinematic viscosity, but the output was only slightly reduced. Cyclodextrin showed an intermediate reduction in aerosol output rate compared to that of glycerin and diclofenac. A 5% CD solution had nearly the same output rate as just a 0.1% DCN in PBS. Ultimately, with increased concentration of cyclodextrin to 20%, there was a point when the output rate was brought to an absolute halt. With glycerin, the rate was drastically slowed, from 5 mg/sec to 3 mg/sec at 20% w/v, but we did not investigate beyond the concentrations necessary for solubilization (Figure 5.4).

In order to establish the most optimized formulation, the solubilization capacity and the effects on output rate must be considered. Glycerin as a cosolvent had a very small effect on output rate, but was an inferior solubilizer to HPBCD. And on the other

hand HPBCD, was a great solubilizer, but negatively affected the output rate. Herein lays the compromise, because the higher loaded solubilities (e.g. 20 CD%) were able to encapsulate much more drug per droplet (i.e higher payload). But this was at the expense of the aerosol generation rate (Figure 5.5). Therefore at a set chamber fill time of 3 seconds, there would theoretically be a reduced amount of drug dispersed. Because of the reduction in output rate at the high CD concentrations, the high drug payload would most likely come from the 1% DCN-1% HPBCD formulation with a 3 sec chamber fill time, because the much higher output rate over compensates for the reduced solubilization as is shown in Figure 5.6, by a net increase in DCN generation over time.

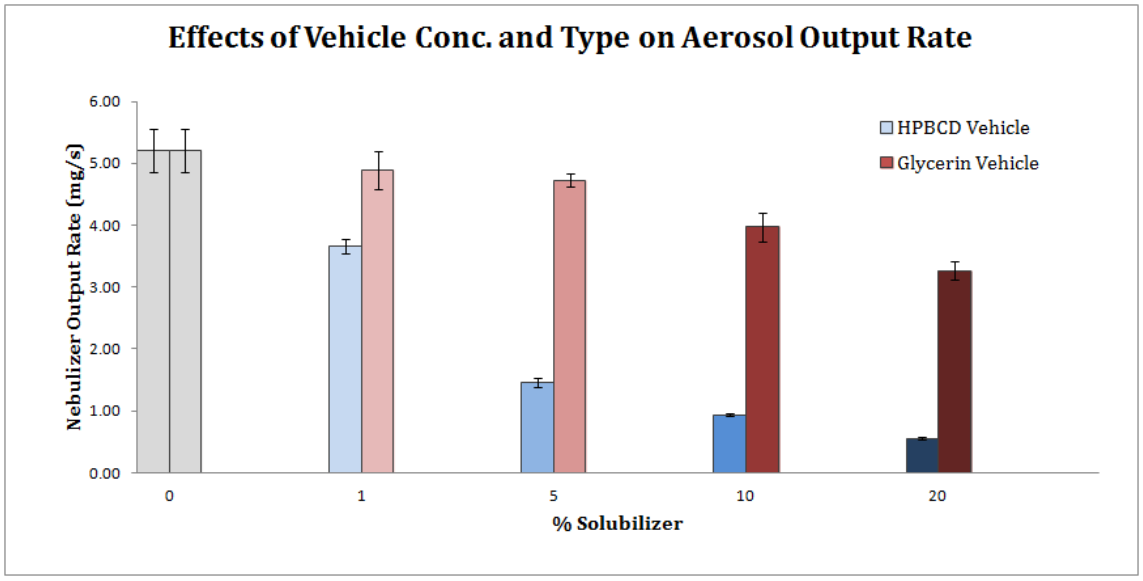


Figure 5.4. Effects of HPβCD and Glycerin Conentration on Aerosol Output Rate

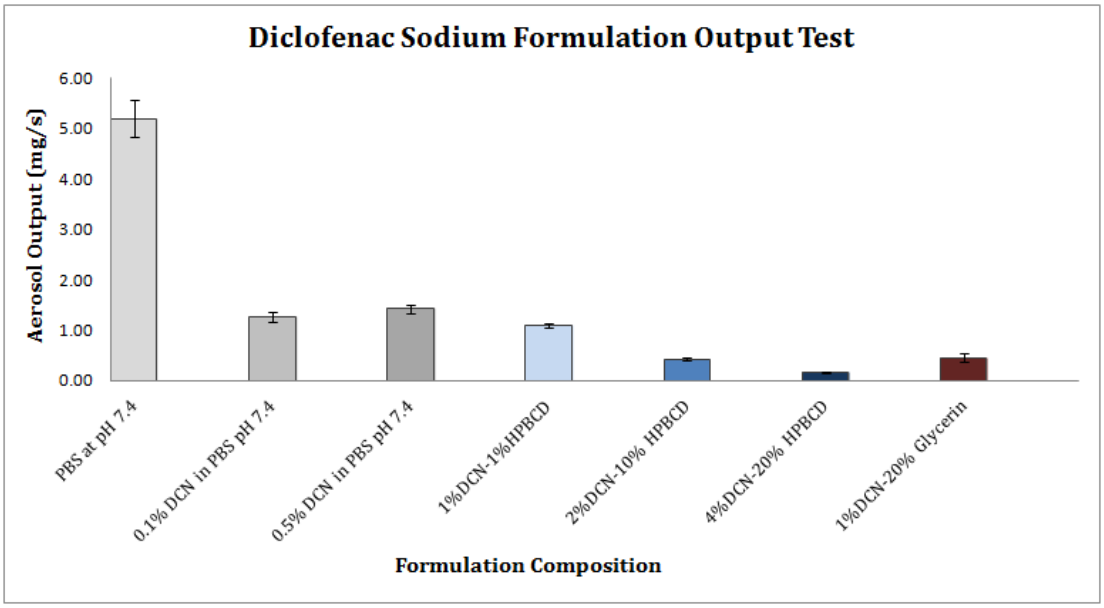


Figure 5.5. Aerosol Output Rate with Diclofenac and Vehicles Combined

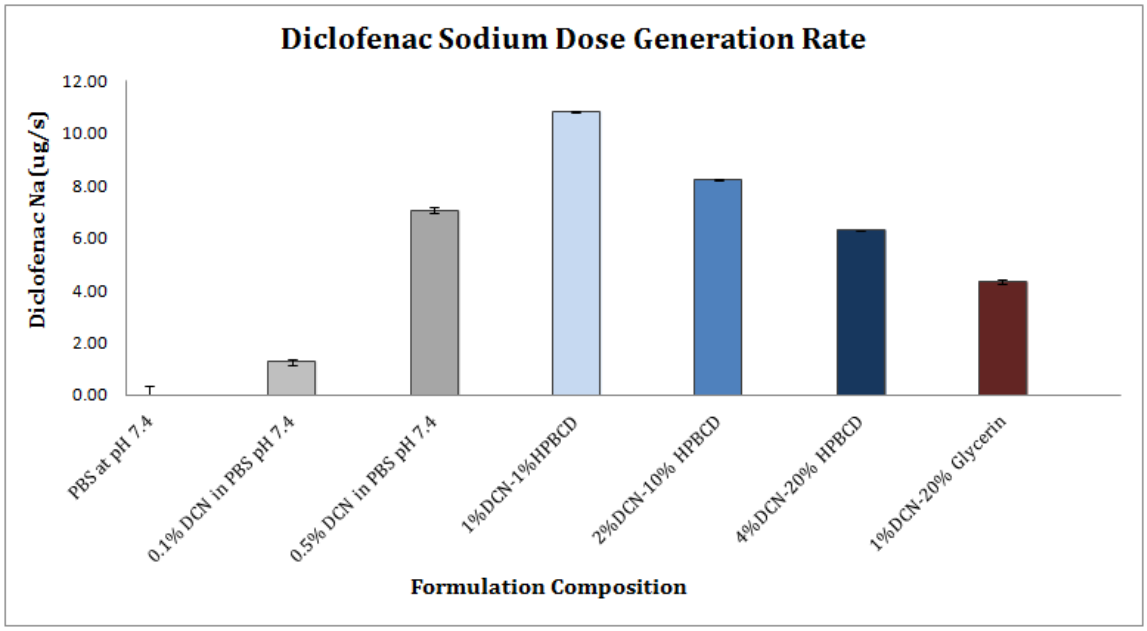


Figure 5.6. Effect of Solubilization Capacity and Output Rate Dictates Maximal Dose Generation Rate.

CONCLUSIONS:

These experiments have been shown to be crucial for the optimization of dosing from this advanced type aerosol delivery system, in that the toroidal vortex dynamics can be physically modulated with the device but the nature of the aerosol being emitted must also be optimized by formulation strategies in advance. Unique challenges are created when attempting to delivery very small volumes of a solution to the ocular surface. Particularly when working with poorly water-soluble drugs or compounds that cannot be formulated at pH in which they are more soluble. And when incorporating solubilizing excipients into aqueous based systems for nebulization special care is needed to ensure that the physic-chemical properties of the solution are not altered to much that characteristics of the generated aerosol are compromise. We found that despite large changes in the viscosity of the solution formulations, it did not have meaningful effect on the droplet size distribution. Furthermore this increased viscosity did have a net negative effect on the nebulizer output rate, demonstrating that there is an upper limit to how much solubilizer can be incorporated and sill form adequate aerosols. This also translates to an up limited of drug solubilization and how much drug can be incorporated into droplets. Finally, a clear trade off exists in the solubilization capacity of the solution, and the aerosol generation rate. Caution must be observed, as high droplet loading may come at the cost of generating much less aerosol, and thus the drug payload. While the effects of the vehicle on aerosol performance were clearly demonstrated here, it is important to re-evaluate the performance when attempting to solubilize other drug molecules because

they will have different physicochemical properties and thus could perform as a vastly different system.

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Chapter 6 : Super-Heated Aqueous Particle Engineering (SHAPE): A Novel Method for the Micronization of Poorly Water Soluble Drugs

ABSTRACT

Super-heated aqueous particle engineering (SHAPE) is a novel particle engineering technology that utilizes the elevated boiling point of pressurized water in order to melt and emulsify hydrophobic drug particles, and then use the newly formed droplets as templates for engineering new particles with different physicochemical properties. When the temperature of an aqueous phase is elevated above the melting point of a drug, the drug quickly melts and forms a low viscosity, immiscible liquid, which is then broken up into nano or micro sized droplets under continuous stirring. The interfacial tension between the droplets and liquid serves to shape them into uniform spherical particles. These droplets can hold their shape as the dispersion is cooled back down below the melting point of the drug. During this cooling stage, either solid crystalline or amorphous microparticles can be formed depending on the cooling rate that is selected. The particles are then collected and can be utilized in many different pharmaceutical applications. In this manuscript we use this particle engineering technique, employing the model drug carvedilol, to investigate the effect that different stabilizers/surfactants exert on the particle size and morphology.

INTRODUCTION

Particle engineering in the pharmaceutical industry has become an essential tool for the fine-tuning and performance of pharmaceutical products, with broad applications. The range of purposes for particle engineering is quite broad and encompasses everything

from reducing particle size, to improving dissolution and enhancing absorption, all the way to producing stabilized particles with optimal characteristics for various routes of administration. Despite the broad utility of particle engineering, the main goal in every situation is to alter the physicochemical characteristics of a raw material in order to overcome the barriers or problems that occur with the: manufacturing, stability, performance, and efficacy of the drug product.

The overwhelming majority of particle engineering technologies come from either of two different classifications, the top-down approach or the bottom-up approach, each having differences in advantages and disadvantages. The top-down approach utilizes mechanical energy and/or shear to break apart particles into smaller, more uniform particles. Oppositely, the bottom-up approach uses a solvent or mixtures of solvents to precipitate particles from a solution. It should also be mentioned that there are also combination techniques, which use precipitation and milling/grinding or homogenization in conjunction in order to achieve the proper particulate product characteristics, such as the NANOEDGE™ and smartCrystal® technologies (1). In general, top-down approaches are time consuming, requiring many long milling steps, and there have been reports of contamination of the final product with fragments of the milling media. In addition, high energy milling can impart amorphous characteristics onto the surface of the particles. This may have negative effects on the stability and/or performance of the final drug product. The major drawback for bottom-up approaches is that these methods require that the engineered material be adequately soluble in a particular solvent (or solvent system), and must be able to be precipitated with another. This may not always be achievable with appropriate pharmaceutical solvents. Furthermore, many of these solvents are toxic and processing materials with them requires additional purification steps. The use of many of these solvents also increases safety risks during

manufacturing, which, in turn, increases production costs and raises additional environmental concerns.

We introduce herein a new, solvent-free approach, which utilizes thermal energy to create a molten-templated intermediate step, for the formation of uniform particles. This technique can be described as being a hybrid of the two main approaches previously mentioned, as this novel approach uses mechanical energy to disrupt and break-up droplets, but a melting step allows for the disruption of the original crystalline structure, which is reformed upon controlled cooling. This reformation of the crystal form offers an additional potential benefit for this processing technology, in that, by altering the cooling or heating profile one can potentially alter and/or select for different polymorphic forms of the active pharmaceutical ingredient (API) (2). The selection of the polymorphic form of pharmaceutical actives is essential as they can have largely different physicochemical properties (3). According to Lee et al, and Ostwald's rule of stages, melting and cooling profiles can be altered to select or screen polymorphs with different stabilities as the molecules are able to transition between forms depending on their energy (4). Although polymorphs can be selected and screened by precipitation processes and other methods, the SHAPE technique offers an all-in-one approach for the production of fine, monodisperse particles, with a potential opportunity to selectively alter their polymorphic form as required by the API and application.

In this article, the processing and post-processing methods, as well as a proposed mechanism for the formation of droplets created in this manner, are discussed in detail. Furthermore, the role of different stabilizers in the formation/stabilization of the engineered particulates and any effect their structure or concentration may have on the generated particles is assessed. Materials and Methods

Materials

We utilized a customized stainless steel pressure vessel was fabricated in house to have a sight glass, pressure gauge and a cooling coil sampling port. Carvedilol was purchased from TCI America (Portland, OR). And we purchased Poloxamer 407, Sodium Carboxymethyl Cellulose and Tween 80 from Spectrum Chemicals (Gardena, CA, USA).

Methods

Processing Parameters for the Production of Carvedilol Particles

Samples were all heated from 40 – 125 ^{o}C under stirring at 200 rpm with a maximal pressure of between 20-25 psi. Samples where then allowed to continue stirring for 5 minutes at 125 ^{o}C /20 psi, then removed from heat and allowed to cool for 30 minutes. (See Figure 6.1 for a detailed discussion description processing technique.)

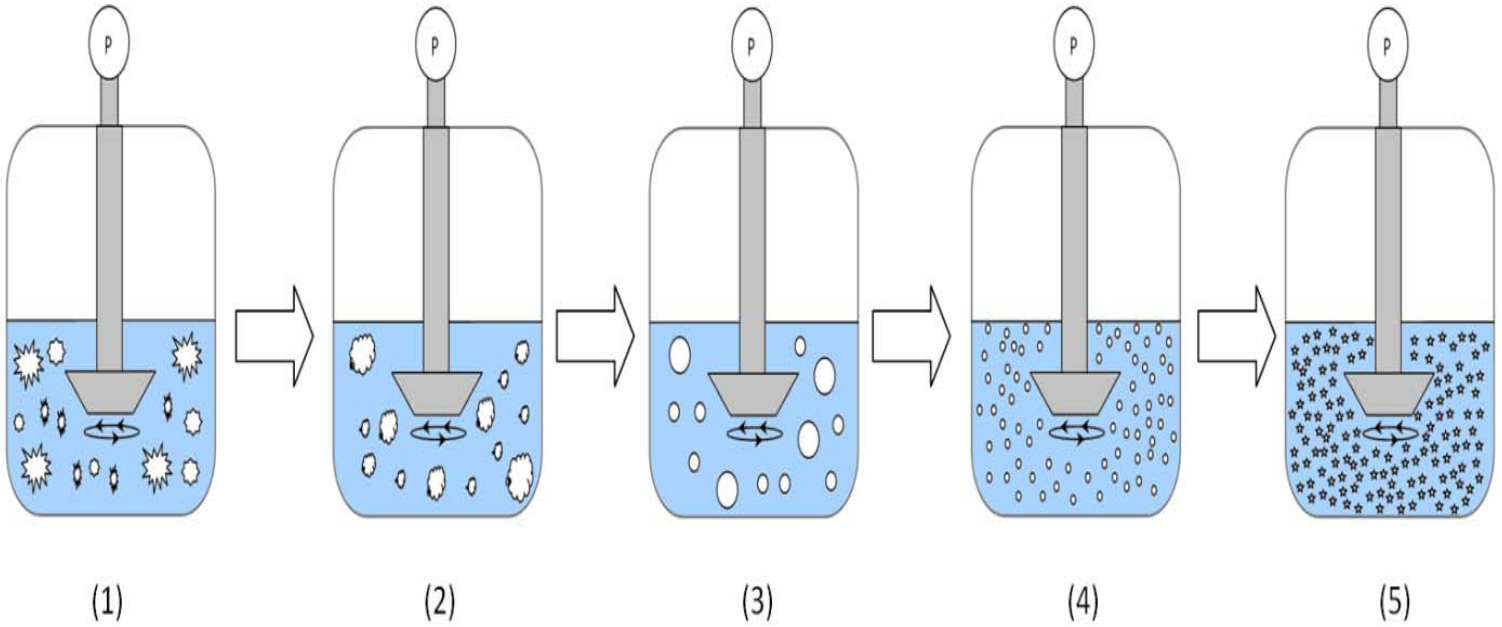


Figure 6.1. **SHAPE Processing Overview and Schematic**: (1) A coarse, poly-disperse suspension of a poorly water-soluble drug (2) Increasing the temperature under pressure, to a point above the melting point of the drug (3) Upon further heating, the viscosity of the drug decreases to a point where a molten drug emulsion forms (4) With continued mixing, the droplet size is reduced to form a uniform emulsion, with dramatically reduced droplet size (5) The emulsion is then allowed to cool back to below the melting point of the drug to form mono-disperse crystals

Table 6-1. Table of Formulations Screened with SHAPE.

Formulation	API	%	Surfactant	%(w/v)
CARV-DI	Carvedilol	0.50	N/A	N/A
CARV-PA	Carvedilol	0.50	Poloxamer 407	0.50
CARV-PB	Carvedilol	0.50	Poloxamer 407	0.10
CARV-PC	Carvedilol	0.50	Poloxamer 407	0.05
CARV-NA	Carvedilol	0.50	NaCMC	0.50
CARV-NB	Carvedilol	0.50	NaCMC	0.10
CARV-NC	Carvedilol	0.50	NaCMC	0.05
CARV-1-PC	Carvedilol	1.00	Poloxamer 407	0.50
CARV-1-PD	Carvedilol	1.00	Poloxamer 407	1.00
CARV-PC-NaOH	Carvedilol	0.50	Poloxamer 407	0.50

Method for Collection of Particles

Upon cooling to room temperature, particles were centrifuged at 4,000 rpm with a Hettick Universal 320R benchtop centrifuge for between 30 and 60 minutes, or until supernatant was clear. The pellet was collected and dried at 35 ^{o}C +/- 5 ^{o}C overnight.

Characterization of Formulations:

Optical Light Microscopy

Freshly processed formulations were equilibrated to room temperature and aliquoted onto glass microscope slides and covered with a cover-slip for visualization. An upright Leica M205 FA Stereoscope was used for imaging of the suspended particles, and digital images were captured using the onboard software.

Dynamic Light Scattering (DLS)

The Dynamic Light Scattering technique was utilized to obtain particle size information with a Malvern Zetasizer ZS. Samples were diluted ~100x with DI water prior to analysis. The Z-average particle size and the polydispersity index (PI) were determined for each formulation using the cumulant fit statistical analysis incorporated into the Zetasizer ver. 7.11 software.

Differential Scanning Calorimetry (DSC)

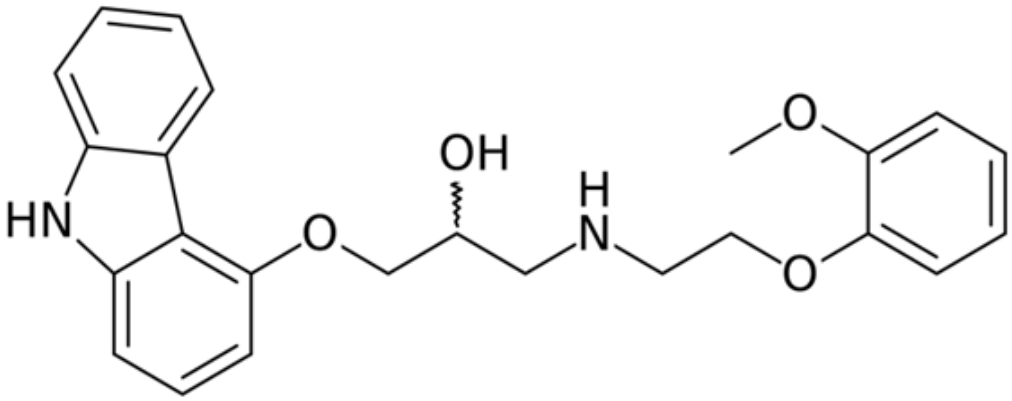
To determine the physical state and thermal properties of the particulates, differential scanning calorimetry was performed on a Texas Instruments DSC Q200 equipped with an auto-sampler. Dried powders were weighed on an analytical balance to be between 1-5 mg and placed in aluminum sealed pans. The heating profile was set to equilibrate at room temperature and ramp from ~25 ºC to 140 ºC at a rate of 10 ºC/minute.

Powder X-Ray Diffraction (PXRD)

Powder X-ray diffraction patterns were captured by using a Spider R-axis X-Ray diffractometer (Rigaku Co., Ltd., Tokyo, Japan). Small agglomerates of powder were attached to a mineral oil coated nylon wire sample holder and placed directly into the aligned X-Ray beam bath. The measurement conditions were as follows: target, Cu Kα; filter, Ni: voltage, 40 kV; current, 40 mA; scanning speed, 1 º/sec, with an exposure time of 10 minutes. Diffraction patterns were collected around a complete rotational axis. This pattern was then converted to 2 dimensional diffractograms and 2-theta angles were plotted against signal intensity in counts per second (Cps).

Scanning Electron Microscopy- (SEM)

Dry powder samples were fixed to SEM stubs using conductive double sided adhesive tape. The samples were sputter coated with 15 nm of platinum/palladium using an ion sputter in a nitrogen atmosphere. The morphological features as well as dimensional characteristics of the powders were visualized at multiple magnifications using SEM (Zeiss Supra 40V, Carl Zeiss Microscopy GmbH, Jena, Germany)



Mp: 114 – 115 °C, pKa= 7.8, LogP=2.74 (at pH 7.0)

Figure 6.2. Molecular Structure of Carvedilol with Physicochemical Properties.

RESULTS AND DISCUSSION

Production of Engineered Particles

Method of Production of Particles and Conditions/Formulation Rationale

Our primary goal in investigating this new particle engineering technique was to determine the effect that different stabilizers have on the nature of the particles generated with this novel technique. It is established that many different excipient stabilizers can play various roles in the production and preservation of nanostructured or micronized particulates (1) . In this preliminary assessment, we chose two polymeric stabilizers with different potential mechanisms. Sodium carboxymethyl cellulose (NaCMC) was selected for its potential to be adsorbed onto the surface of the newly formed particles and to possibly disrupt crystal growth or to serve as a protective to prevent agglomeration due to steric effects. Secondly, we chose poloxamer 407 because it can also be adsorbed onto the surface of newly formed particles, but as an amphiphilic polymer it can serve as a surfactant to reduce the interfacial tension at the interface between the particles and the aqueous phase (2). In addition, it could possibly assist in the emulsification of the molten API droplets.

The main processing variables/conditions for producing nano/micro-sized particles using SHAPE, are temperature, mixing rate and time. While these parameters are singularly simple, they can be independently altered to create a near infinite number of Time-Temperature-RPM Profiles for processing materials. For these experiments, instead of altering processing conditions we opted to select a basic temperature-time-mixing profile in order to assess the contributions that surfactants have on the process.

For this profile, the suspension was mixed at around 400 rpm, which can easily be achieved with a magnetic stir bar. We chose a moderate heating rate that can be achieved using a stirring hot plate. For the maximal processing temperature, we selected 125 ^{o}C, which is approximately 5 ^{o}C over the melting point of Carvedilol. The temperature was held for 5 minutes after the visual confirmation that an emulsion had formed. The mixture was then cooled it back down to room temperature over a 30 minute period under constant mixing.

Method for Collection Particles:

The method used to harvest the engineered particles can have a tremendous effect on the characteristics of the powders produced. This post-processing step is crucial and very dependent on what the dosage form selection or application the API will be used for. In this study we selected a basic centrifugation method because the only concern was investigating the characteristics of the particulates produced by this engineering method. Due the differences in the particle size distributions produced by SHAPE, differing amounts of centrifugation may be required to pellet different solid materials. It was noticed that for carvedilol, the majority of the samples produced herein were able to be pelleted in an under 1 hour at 4000 rpm.

Particle Size and Morphology

Optical Light Microscopy

In order to assess if the particle size was indeed reduced during the processing, we quickly added a few drops of the suspension to a microscope slide and captured images. Figure 6.3 shows the raw, unprocessed carvedilol suspended in a 0.05% poloxamer 407 solution. Crystals are present in variety of sizes and shapes and some are quite large, on the order of 50 μm (by length). As can be seen in Figure 6.4 , the freshly processed material from SHAPE is drastically reduced in particle size and the particle geometry is much more homogeneous.

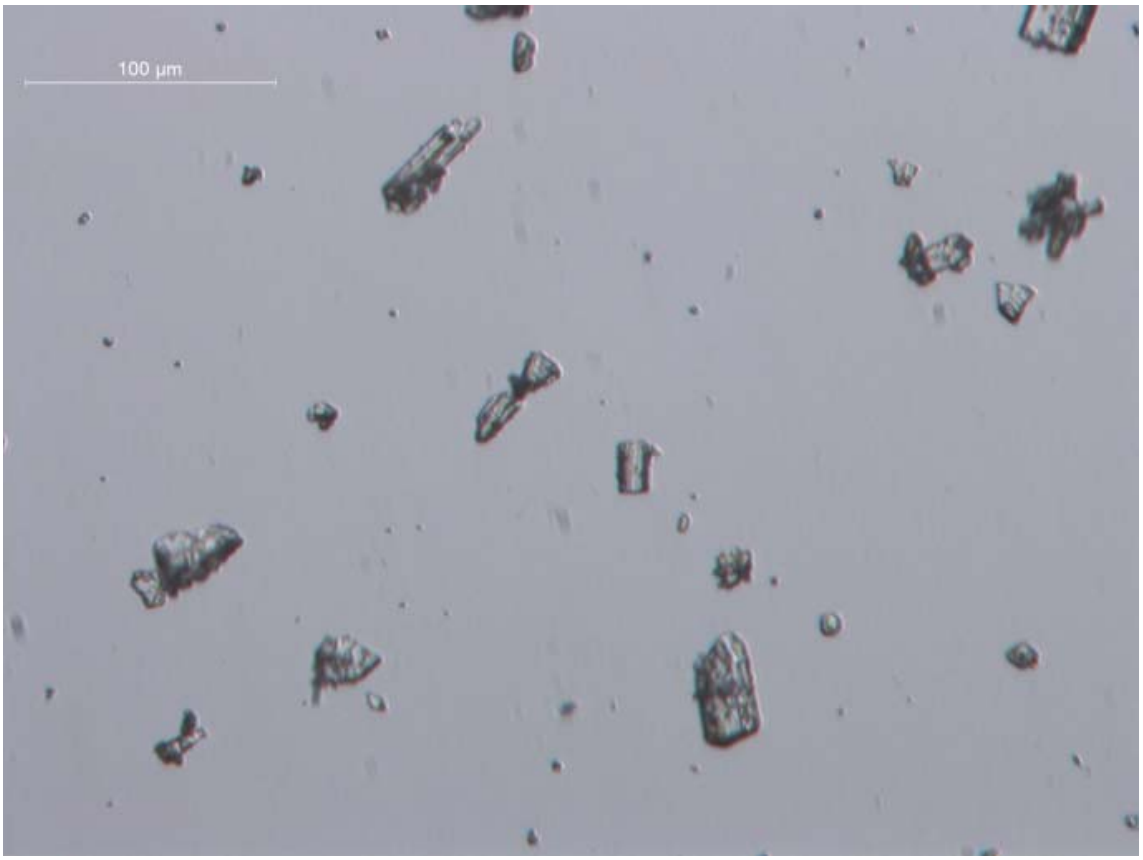


Figure 6.3. Light Microscope Image of Raw Unprocessed Carvedilol Suspended in 0.05% poloxamer 407 solution.

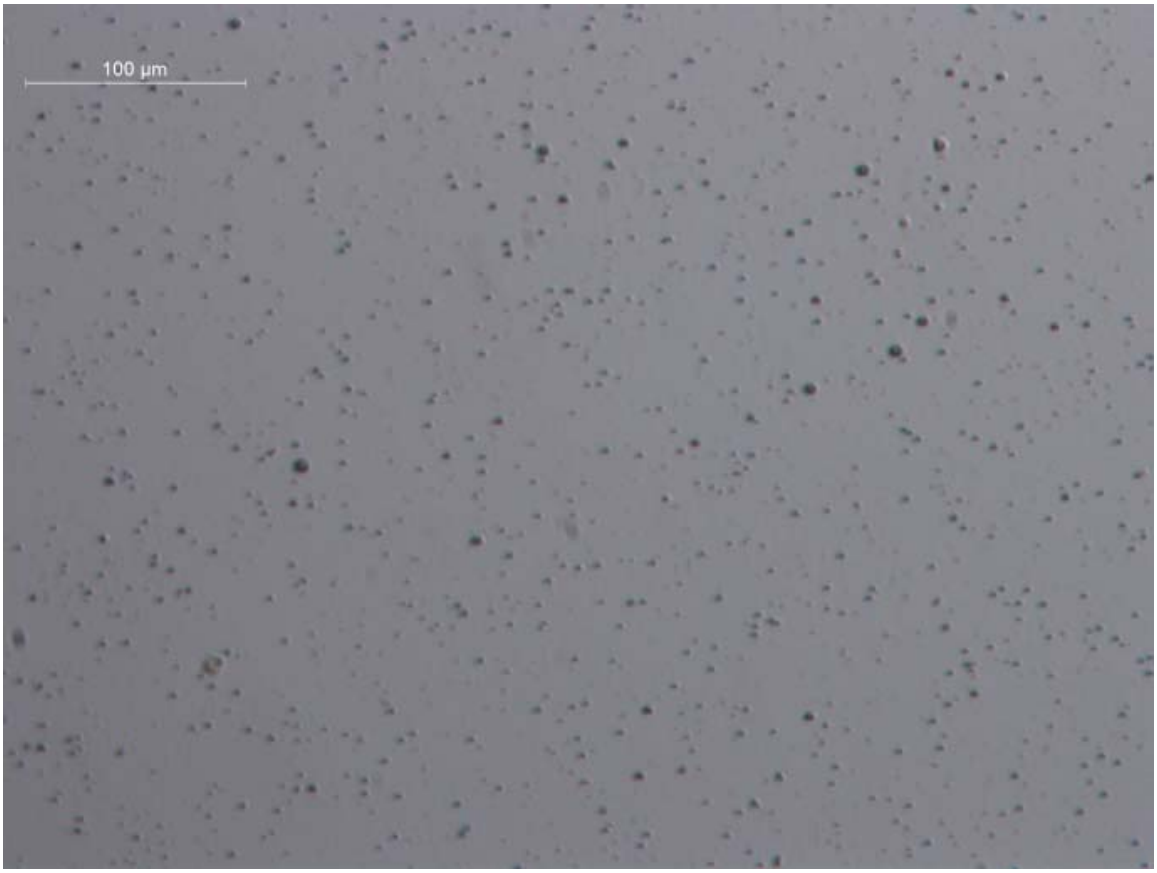


Figure 6.4. Light Microscope Image of SHAPE Processed Carvedilol (w/0.05% poloxamer 407)- Taken immediately after processing.

Dynamic Light Scattering (DLS)

Upon equilibrating freshly prepared suspensions to room temperature, the particle size distributions were assessed using DLS. DLS is additionally useful for determining the short range physical stability of the formulations. Freshly engineered particles will be cooling from a molten state; therefore they are more susceptible to increased coalescence and 'droplet' aggregation, making monitoring their short-term stability essential. As can be seen in Table 6-2, the Z-avg particle size for each of the formulations was in the micron range, demonstrating that the total particle size distribution (PSD) of the raw material can be reduced. However, some of the formulations exhibited a large poydispersity index (PI). This is an indicator of either particle aggregation or precipitation. Furthermore, a clear difference can be seen between that of the suspension produced with poloxamer 407 vs that of NaCMC. The particles produced from NaCMC were all larger and had broader size distributions. The two formulations with the lowest particle size were both with 0.05% poloxamer 407. Both of these produced particles with a Z-avg of less than one micron with a narrow PI. As can be seen in the PSD distributions, the PC formulations displayed a smaller PI, and can thus be considered more homogeneous or uniform (example distribution in Figure 6.5). Based off of this data, the PC formulations appear to the most optimal candidate. They resulted in very small uniform particles that remained in stable suspension over the 30 minute cooling period. This short range stability is important because it allows for a more broad range of post processing techniques. The other formulations appear to either coalesce during the cooling phase or they are forming agglomerates upon equilibration, based off of the larger sizes and very high PI. Future studies will be conducted to assess different post processing techniques and to further analyze the short term stability of these engineered particles.

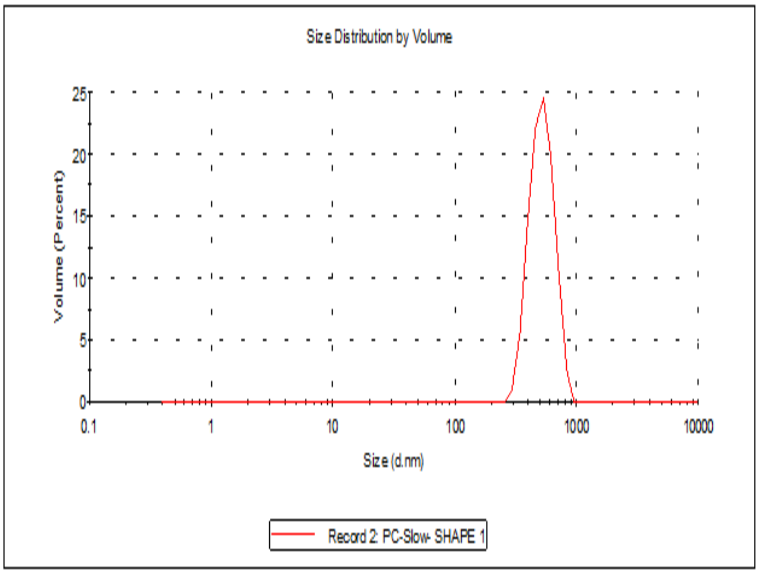


Figure 6.5. Example Dynamic Light Scattering Particle Size Distribution for SHAPE Processed Carvedilol. Formulation: 0.5% Carvedilol - 0.05% poloxamer 407

Table 6-2. Dynamic Light Scattering Particle Size Data and Qualitative Description of SHAPE Processed Carvedilol Formulations.

Formulations	Z-avg(nm)	Polydispersity Index (PI)	Mode (mono/multi)
CARV-DI	3534	0.624	Monomodal/Coarse
CARV-NA	1.57E+04	0.221	Very Coarse
CARV-NB	4334	0.135	Multimodal/Coarse
CARV-NC	4908	0.055	Monomodal/Coarse
CARV-PA	465.4	0.407	Polydisperse
CARV-PB	790.4	0.795	Polydisperse
CARV-PC	539.6	0.265	Monomodal
CARV-1-PC	496.8	0.231	Monomodal
CARV-PC-NaOH	224.9	1	Multimodal/Polydisperse

Scanning Electron Microscopy

Scanning electron microscopy was utilized for few different purposes in this investigation. Most importantly, because we were analyzing and comparing carvedilol raw material to that of the processed material, it was expected for there to be a dramatic difference in their physicochemical properties and for this reason several different methods were used for comparison. The bulk, raw material shown in Figure 6.6, is characterized by large, crystalline particles, with sharp edges. The particles appear to be in a broad range of sizes, up to 100 μm, and all appear to be of the same particle morphology. Then, when comparing the raw material to the SHAPE processed material Figure 6.7, at the same magnification, it can easily be seen that the SHAPE material has formed much larger particle agglomerates with irregular geometry. In the SHAPE sample, these large agglomerates have a show a very rough surface. Upon increasing the magnification in Figure 6.8, discrete individual platelet-type, disk particles can be visualized. These individual particles are relatively uniform and look as if they are packed tightly to form the larger secondary particles (i.e. agglomerates). This is almost certainly due to the post-processing centrifugation step, as the suspension was spun down into a pellet for drying and collection. Based off these SEM images, the particles appear to be about 2 or 3 microns in diameter, but because they are disk like particles and are laying flat it is difficult to assess their thickness. These images confirm the micronization process was successful and support the findings from light microscopy. The DLS sizing indicated that these particles were submicron; however, DLS measurements require a few assumptions based off of particle geometry, and because these particles were found to be plate-like, the reduced reliability of the DLS data must be considered.

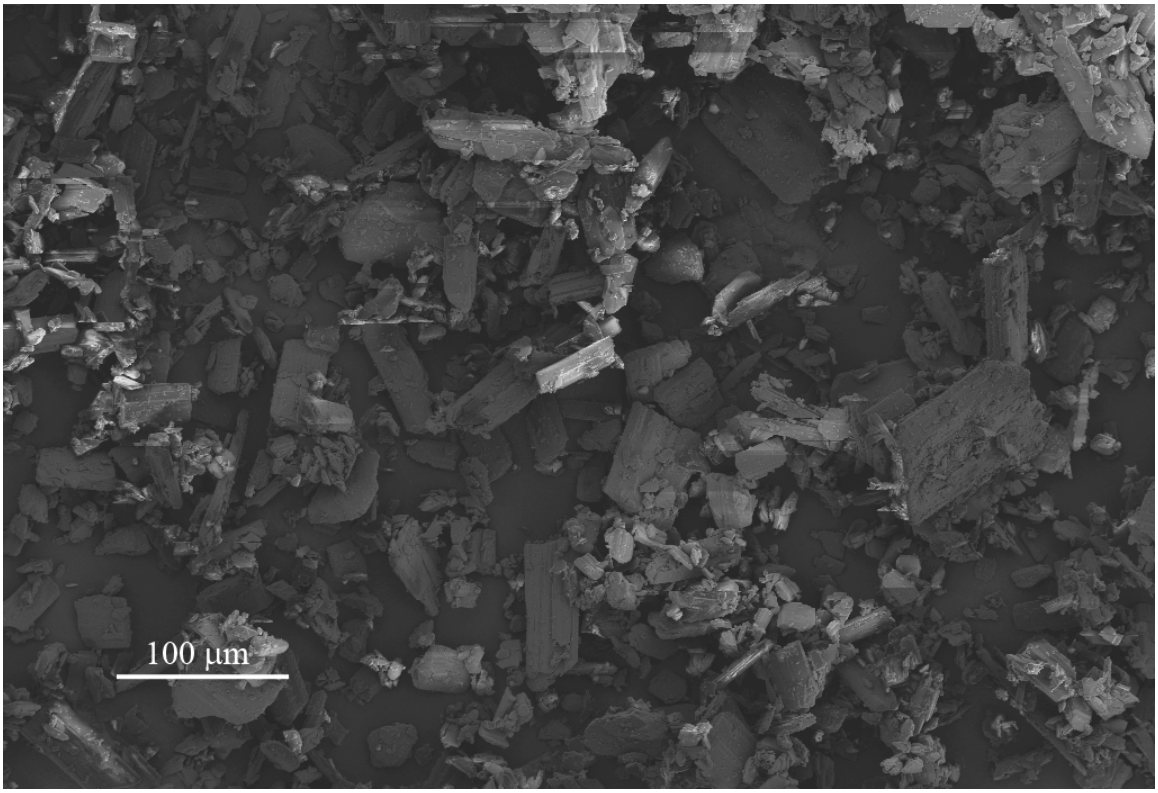


Figure 6.6. Scanning Electron Micrograph of Raw Unprocessed Carvedilol.

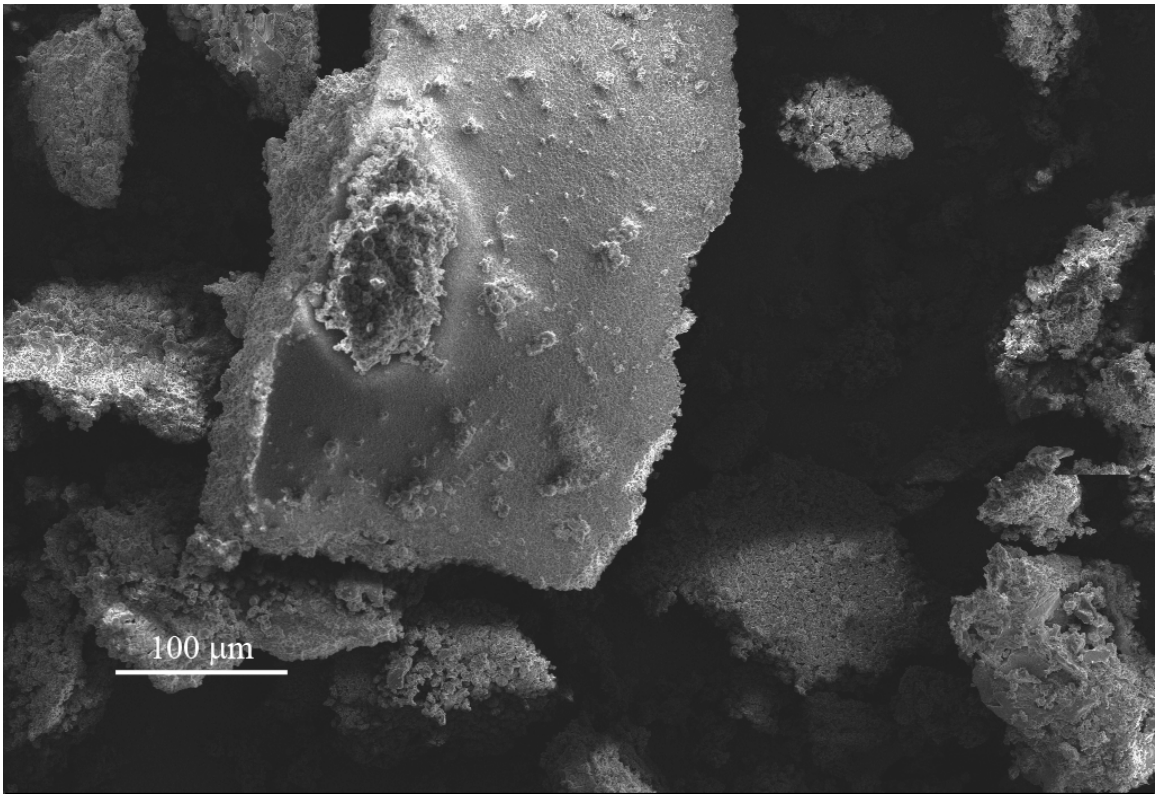


Figure 6.7. Scanning Electron Micrograph of SHAPE Processed Carvedilol Microparticle Agglomerates. (Formulation, CARV-PC: 0.5% Carvedilol-0.05% poloxamer 407)

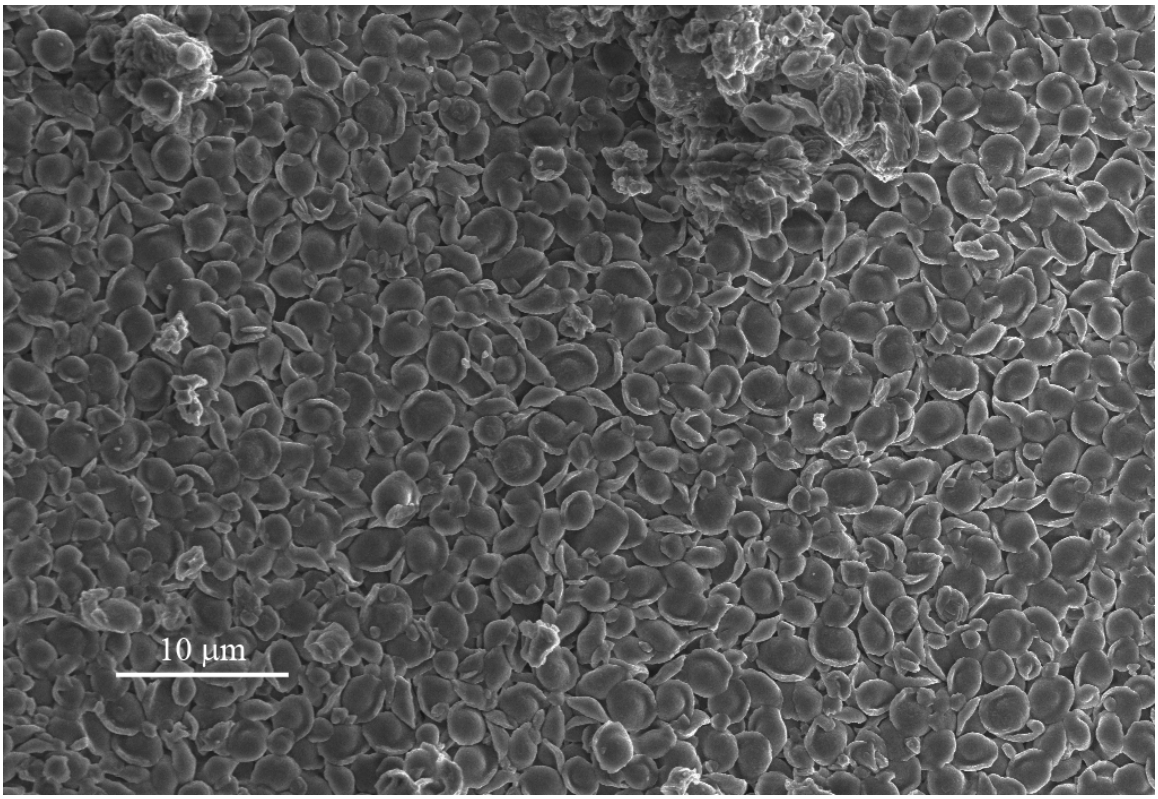


Figure 6.8. Scanning Electron Micrograph of SHAPE Processed Carvedilol Microparticles Under Higher Magnification.(Formulation, CARV-PC: 0.5% Carvedilol- 0.05% poloxamer 407)

Physicochemical Properties of SHAPE Processed Carvedilol

Crystalline Characteristics

The PXRD diffraction patterns for raw, unprocessed carvedilol were compared to that of the SHAPE processed material. The raw carvedilol has several major distinct diffraction peaks appearing at 2-theta angles of: 5.8 ,11.6 & 14.8. These peaks confirm that the starting material is the Polymorphic Form II. Several groups have extensively studied this polymorph and it is well-characterized (3). Upon processing carvedilol with SHAPE, the PXRD patterns reflect a definite change in the crystal morphology. Each batch of processed carvedilol exhibited a near identical diffraction pattern. Most noticeable the disappearance of the 2-theta peak at 5.8 and the emergence of 2-theta peak at ~8.3. The main peaks that appear in these diffractograms are highly similar to that of the Form III polymorph with having nearly all of the same main peaks. However, in order to be certain of this categorization, further analysis would need to be conducted. Furthermore, while comparing the peaks between the processed and unprocessed materials, it is noticeable that the peak intensities of the processed material are smaller than that of the raw material. This decrease in signal intensity is most likely attributed to the dramatic reduction in particle size and/or increased noise due to a relative increase mounting adhesive.

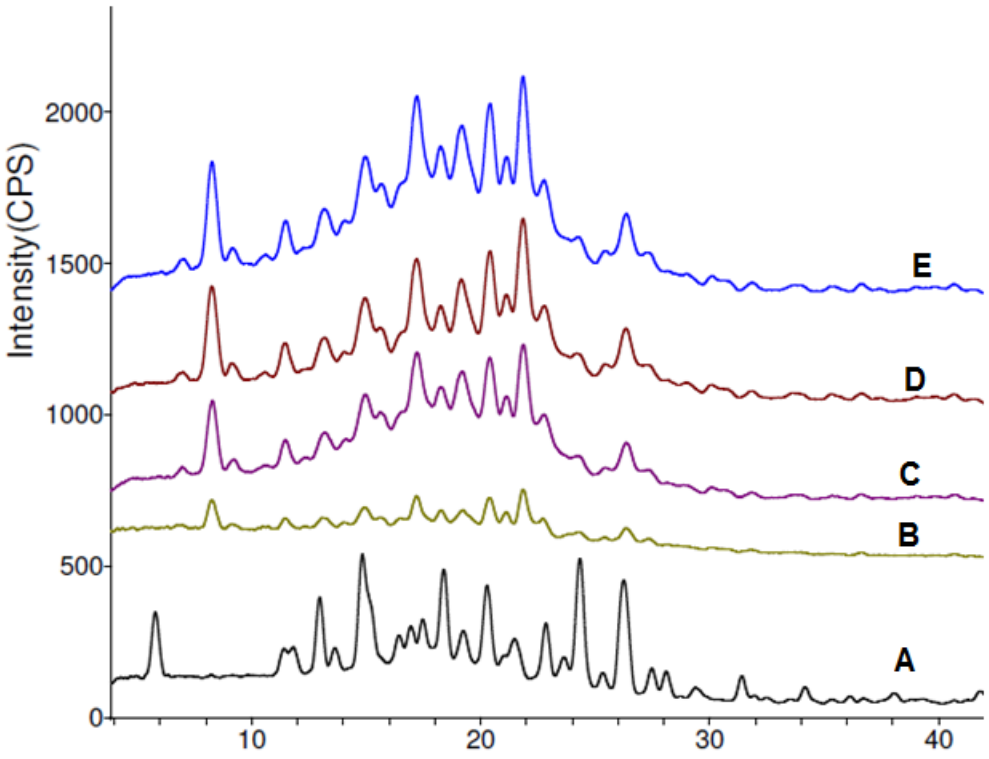


Figure 6.9. PXRD Diffractograms of Raw and Processed Carvedilol. A) Neat Carvedilol before Processing. B) Neat Carvedilol Processed with only Deionized Water. C) 0.5% CARV- 0.5% Poloxamer 407. D) 0.5% CARV- 0.05% Poloxamer 407. E) 1% CARV- 0.05% Poloxamer 407

Physicochemical Properties of SHAPE Processed Carvedilol

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Thermal Behavior

When investigating the possible occurrence of different polymorphic crystal forms it is important to assess the thermal behavior of the materials. We noticed a marked difference in the thermal behavior between that of the raw and processed materials. Most notable was the reduction in melting point of carvedilol in the processed

material Figure 6.10. The melting point of raw CARV Form II was found to be approx 114 ^{o}C, which is in agreement with literature values (3). While the processed materials showed a roughly 20 ^{o}C drop in melting point. This drop in melting point is attributed to the presence of a low melting point polymeric surfactant (poloxamer 407), however the possibility of eutectic formation cannot be ruled out. But because the physical mixture of carvedilol and poloxamer material showed the same drop in melting point, this drop in melting point it is most likely due to the poloxamer melting and beginning to dissolve the carvedilol within the sample pan. Furthermore, it should also be noted that the melting endotherms for the processed particles show more broadening compared to the neat carvedilol endotherm. This broadening and melting point suppression could be attributed to the Kelvin effect described in the Gibbs-Thomson equation, as these particles have a different surface geometry and are much smaller than the raw material, as well as a portion of the material is sub-micron (4, 5). In addition, the broadening and melting point suppression suggests that there is residual poloxamer remaining in the processed powder blend. This can be explained by the nature of the particle collection technique and the experimental setup. In order to collect the particles, they were centrifuged and dried from the surfactant solution; it is likely that an unavoidable film of poloxamer was retained in the powder upon drying. Previously, others have described this occurrence with other surfactants that interact with a nanocrystal surface (6). With these considerations the findings from the DSC thermograms support the findings from the PXRD data that indicates the formation of a possible different crystal form. In addition, the presence of clear endotherms confirms that the particles undergo recrystallization and exist in crystalline form as a dry powder. Further experimentation will be needed to provide more details about the nature and kinetics of the recrystallization process.

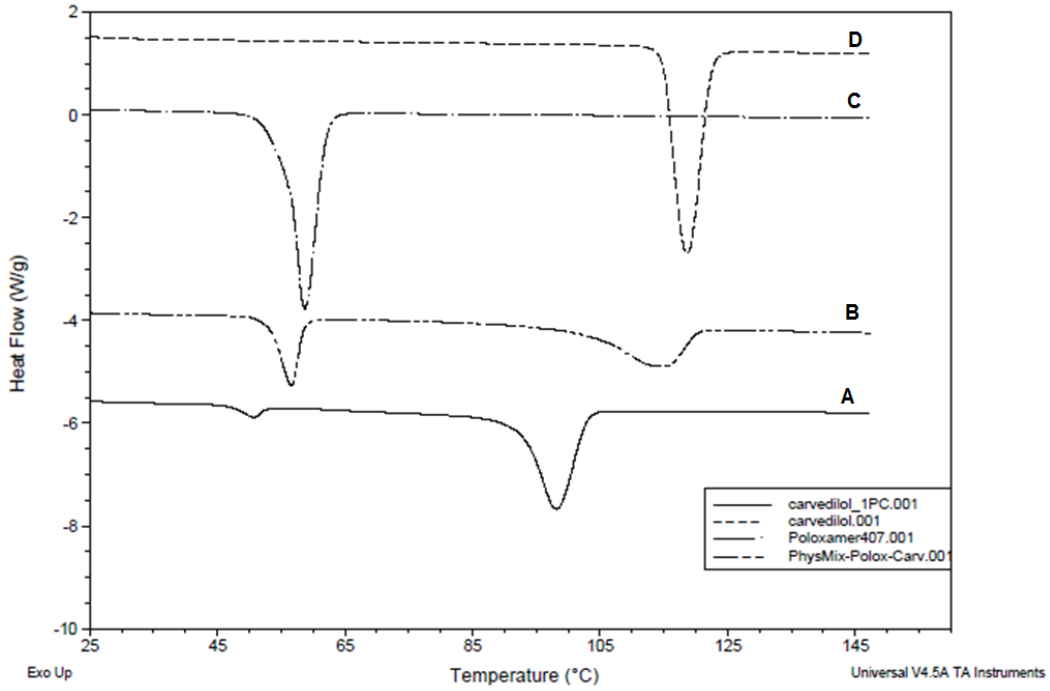

Figure 6.10. DSC Thermograms of Raw Materials and Engineered Powders. A) 1% Carvedilol- 0.05% Poloxamer 407. B) Carvedilol and Poloxamer 407 Physical Mixture. C) Neat Poloxamer 407. D) Neat Carvedilol

CONCLUSIONS

In this investigation we determined that SHAPE is a viable method for the production of fine particles with altered morphology and physicochemical characteristics. It was also concluded that the selection of surfactant/stabilizer can have a tremendous effect on the characteristics of the particles generated. Poloxamer 407 was found to be a superior stabilizer compared to NaCMC, at both aiding the reduction of particle size and the prevention of agglomeration of newly formed particulates. Finally, while the technique was successful at reducing particle size and altering particle morphology, much more research needs to be conducted in order to further optimize the processing parameters to engineer specific particles for different applications and to gain further understanding of some of the underlying mechanisms in this complex dynamic system.

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Chapter 7 : Concluding Remarks

DISSERTATION CONCLUSION

Studies utilizing aerosol loaded toroidal vortices for ocular drug have shown their ability to deliver precise small volumes of liquid in a tunable and reproducible manner. The use of this technology enables characteristics that are not currently attainable with existing technologies. Some of these features include: the ability to readily control the geometric dimensions of the emitted aerosol plume, enhanced control and tunability of the emitted aerosol characteristics, and the ability to finely tune the velocity of the aerosol.

Investigations comparing toroidal vortex velocity and deposition, showed that by controlling the translational velocity of toroidal vortex one could directly control the amount of drug that is deposited onto a surface. Furthermore, by increasing the aerosol concentration or the concentration of the active ingredient in the nebulized solution the deposition efficiency can be directly increased, allowing for dosing over a wide range of therapeutic concentrations. Other investigations revealed that use of an electronic solenoid actuator offers high degree of control of the aerosol velocity and these vortices can be emitted at a desired speed by adjusting the energy used to actuate the membrane. In addition, it was found that the forces generated by these impacting toroidal vortices are far below that of which is normally experienced with standard eye drop solutions, thus ensuring that administration will be comfortable and patient friendly.

The research conducted and outlined here in this dissertation has demonstrated the feasibility of the use of aerosol loaded toroidal vortices for topical ocular drug

administration. Furthermore, when combined with other formulation strategies, the use of this technology can be applied to a very wide range of therapeutic agents and potentially be useful in solving some of the many problems are faced in modern ocular drug delivery.

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